7th TriNet Meeting – RECOOP Annual Project Review Meeting
Hotel Gellert
Budapest, Hungary
October 6 – 9, 2016
Agenda of the 7th TriNet Meeting - Annual Project Review Meeting

Venue: Annual Project Review Meeting on October 7 - 8, 2016 in Hotel Gellert, Budapest.

Arrival on October 6 (Thursday) until 6 pm and
Departure on October 9 (Sunday).

Access to the Spa on Friday late afternoon and Sunday morning free of charge.

October 6, 2016

Arrival until 6 pm.

8:00 pm Buffet Dinner – Panorama Room

Registration desk at Tea Saloon Foyer, time interval for registration 3pm-7pm

October 7, 2016

Registration desk at Tea Saloon Foyer 7:30 am – 12 pm

8:30 – 11:00 am Plenary Session Tea Saloon

8:30 – 9:00 am Review of RECOOP activities

What I learned from the “HFHS diet induced prediabetic, obese elderly rats treated with Liraglutide (Victoza) and Metformin” study.
Sandor G. Vari Director, International Research and Innovation in Medicine Program
Cedars-Sinai Medical Center, Los Angeles, CA, USA & President of the RECOOP HST Association

9:00 – 10:00 am 3 Keynote Speakers

09:00 – 09:20 Connection between low grade inflammation and development of leptin and insulin resistance in animals on HFHS diet
Marija Heffer, Department of Medical Biology and Genetics, Laboratory of Neurobiology
Faculty of Medicine, University Josip Juraj Strossmayer Osijek, Croatia

09:20 – 09:40 Project plan for clinical studies on the mechanism of obesity induced insulin resistance
Tamas Tabi, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary

09:40 – 10:00 Obesity and pregnant uterine contractility: molecular and functional investigation of the roles of leptin, adiponectin and kisspeptin in rats (project plan)
Robert Gaspar, Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary
10:00 – 11:00 RECOOP Research in Progress

Chairperson:
Marija Heffer
Robert Gaspar
Tamas Tabi

10:00 – 10:15 Research activity in 2015-2016 at the Institute of Cell Biology, NAS of Ukraine: achievements, problems and perspectives of development within RECOOP-HST Network
Rostyslav Stoika, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, NAS of Ukraine,

10:15 – 10:30 Leptin receptor is present in neural and adrenal gland stem cells, allowing stress influence through leptin signaling
Srečko Gajović, University of Zagreb School of Medicine, Croatian Institute for Brain Research, Croatia

10:30 – 10:45 Triggered liver regeneration in portal vein ligation models
Attila Szijjártó, Hepato-Pancreatico-Biliary Surgery Research Center Hungary, 1st Department of Surgery, Semmelweis University, Budapest, Hungary

10:45 – 11:00 The Role of Skeletal Muscle Contractile Duration on Cognitive Functions and Health Outcomes
Oksana Zayachkivska, Physiology Department, Danylo Halytsky, Lviv National Medical University, Lviv, Ukraine

11:00 – 11:30 am Coffee Break – Gallery - Opening of Art and Sciences Exhibit
Semmelweis Publishing, “Resourceful Medical Science - Basic Disciplines on Posters”. RECOOP – Sciences and Arts

11:30 – 12:30 pm Plenary Session Tea Saloon
Presentation for the 2017 CMJ RECOOP Special Issue Manuscripts: 4 manuscripts each for 12 minutes, panel discussion for 12 minutes.
Chairpersons:
Oksana Zayachkivska
Rostyslav Stoika
Srečko Gajović

Sex Differences in the Oxidative Stress Level and Antioxidative Enzymes Activity in Obese Pre- Diabetic Elderly Rats Treated with Metformin or Liraglutide
Anita Cosic, University Josip Juraj Strossmayer Osijek, Faculty of Medicine, Department of Physiology and Immunology, Osijek, Croatia,

The Effect of Environmental Enrichment on Retinal Damage After Prenatal Stress
Timea Kvarik & Tibor Ertl Department of Obstetrics and Gynecology, Neonatology, Medical School, University of Pecs, Pecs, Hungary

Effects of valerian root extract and commercial “valerian-drink” on anxiety-like behavior and oxidative status in rats
Emese Domonkos, Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia
Tissue-Protecting Effect of Antioxidants Selenomethionine and D-Pantethine Towards Doxorubicin Toxicity in B16 Melanoma-Bearing Mice
Rostyslav R. Panchuk, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology NAS of Ukraine

12:30 – 01:15 pm Plenary Session – Summary of the Poster Presentations
8 posters for 5 minutes, no questions and discussions.

Chairpersons:
Katarina Sebekova
Ines Drenjancevic
Tibor Ertl
Imre Fehervari
Andrea Gaspar Suranyi

Renal histopathological alterations in diet-induced diabetic, obese elderly rats treated with Liraglutide or Metformin
Peter Boor, Institute of Pathology, University Clinic of the RWTH Aachen, Aachen, Germany

Analysis of rat plasma samples of elderly rats with HFHS diet and treated with Liraglutide (Victoza) and Metformin.
Boglarka Donczo, University of Debrecen, Research Centre for Molecular Medicine, Horvath Laboratory of Bioseparation Sciences, Debrecen, Hungary

Sex-specific development of type II diabetes – model for pathogenesis
Mtiaj Fenrich, J. J. Strossmayer University of Osijek, Faculty of Medicine Osijek, Osijek, Croatia

Adiponectin receptors in the brain of rats on HFHS diet
Senka Blažetić, J. J. Strossmayer University of Osijek, Department of Biology, Osijek, Croatia

Cellular models of insulin and leptin signaling
Marianna Pap, Department of Medical Biology, Medical School, University of Pecs, Hungary

High fat/sugar diet causes sex specific reactive gliosis in the brain of Sprague Dawley rats
Milorad Zjalic Faculty of Medicine, Josip Juraj Strossmeyer University of Osijek, Croatia

Sex differences and sex hormones role in aortic reactivity to acetylcholine in Sprague-Dawley rats with and without streptozotocin induced diabetes mellitus
Zrinka Mihaljević, Department of Physiology and Immunology, Faculty of Medicine Josip Juraj Strossmayer, University of Osijek, Croatia.

Diet induced changes of kisspeptin receptor expression in fatty tissues of elderly rats treated with Liraglutide (Victoza) and Metformin.
Eszter Ducza, Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

01:15 – 1:50 pm Goblein room RECOOP Posters discussion
Poster size Height 120cm Width 90cm
Katarina Sebekova
Ines Drenjancevic
Tibor Ertl
Imre Fehervari
Andrea Gaspar Suranyi

1:50 – 2:00 Summary of the Posters discussion
Katarina Sebekova
October 8, 2016

8:30 – 10:30 am Plenary Session Tea Saloon

8:30 – 8:45 am Summary of RECOOP General Assembly and RECOOP Project Review
Sandor G. Vari

8:45 – 11:00 am Presentations for the 2017 CMJ RECOOP Special Issue
6 manuscripts each for 12 minutes, panel discussion for 10 minutes.

Chairpersons:
Zora Krivosikova
Andrijana Muller
Srećko Gajović

Pregestational BMI: how does it affect the perinatal outcome in diabetic women?
Andrea Gaspar Suranyi, Department of Obstetrics & Gynecology, University of Szeged, Hungary

4-thiazolidinones as novel anticancer agents for treatment of melanoma cells
Nataliya Finiuk, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine

Identification of myoelectric signals of pregnant rat uterus: new method to detect myometrial contraction
Kalman F. Szucs, Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

Differential pro-apoptotic effects of novel 4-thiazolidinone derivatives in human glioma cells
Lesya I. Kobylinska, Department of Biochemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Neuroprotective effects of 4-thiazolidinone derivatives in rat model with MPTP-induced Parkinsonism
Olesya Poshyvak, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine, Department of Pharmaceutical, Organic and Bioorganic Chemistry

Blood Serum 48 kDa Form of the Unconventional Myosin 1c as a New Potential
Rostyslav S. Stoika & Yuriy Y. Kit, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine.

11:00 – 11:30 am Coffee Break

Gallery Sciences and Arts Exhibit
Gobleín room RECOOP Posters discussion

11:30 – 12:00 pm Tea Saloon Plenary Session Poster presentations’ oral summary
5 posters will be presented for 5 minutes, no questions and discussions.
Chairpersons:
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Ines Drenjancevic
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Soluble fms-like Tyrosin Kinase 1 (sFLT-1) to Placental Growth Factor (PIGF) Ratio as Possible Indicator for Severity of Preeclampsia
Andrijana Muller Department of Gynecology and Obstetrics, University Hospital Center Osijek, Croatia

B16F10 Murine Melanoma as A Promising Model for Simultaneous Evaluation of Therapeutic Efficiency and Side Effects of Novel Anticancer Drugs
Lilya Lehka, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Mebrofenin transport test for the quantification of hepatic function following portal vein ligation
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Modulatory Effect of Γ-Fe₃O₃ Nanoparticles, Functionalized with Ascorbic Acid, On Antitumor Activity of Doxorubicin In Vitro
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HPLC analyses of iron nanoparticles planned to conjugate with anticancer drugs, but it was not proven
Anita Sztojkov-Ivanov, Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

12:00 -01:00 pm Goblein room RECOOP Posters discussion
Poster size Height 120cm Width 90cm

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Ines Drenjancevic
Tibor Ertl
Imre Fehervari
Andrea Gaspar Suranyi

01:00 – 02:00 pm Buffet Lunch – Panorama Room

02:00 – 3:15 Presentations for the 2017 CMJ RECOOP Special Issue
4 manuscripts oral presentation each for 12 minutes, one manuscript for poster presentation, panel discussion for 15 minutes

Effectivity of health screening in Hungarian General Practitioners’ Communities: role of medical auxiliaries in prevention
Istvan Marton Kiss, University of Szeged, Faculty of Medicine, Department of Health Economics, Szeged, Hungary

4-Thiazolidinone-based Derivatives Rescue TNFα-Inhibited Osteogenesis in Mouse Mesenchymal Precursor Cells
Khrystyna Malysheva, Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine

To get or not to get supplementation during malignant diseases
Dorottya Muhl, Cancer Cente, Semmelweis University Budapest, Hungary

Changes of Lipid Risk Factors in Women During and After Transition to Menopause.
Jan Pitha, Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Poster: Ultrastructural testicle of streptozotocin-induced diabetes of rat
Dzhalilova Elvira, Department of normal anatomy, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

03:15 – 04:00 pm Breakaway Sessions

Tea Saloon - Common Mechanism Diseases
Chairperson: Sandor G. Vari

Obesity-induced insulin resistant rats: Interrelation between changes in leptin and cholesterol concentrations and key characteristics of glutamate- and GABA-ergic neurotransmission (project plan)
Tatiana Borisova Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine

Coffee Saloon - NanobioTechnology
Chairperson: Rostyslav Stoika

04:00 – 04:30 pm Coffee Break
Gallery Sciences and Arts Exhibit
Goblein room RECOOP Posters discussion

04:30 – 06:15 pm Plenary Session Tea Saloon - 2017 CMJ RECOOP Ukrainian Special Issue Manuscripts
7 manuscripts each for 12 minutes, panel discussion for 10 minutes.

Chairpersons:
Marija Heffer
Lesya I. Kobylinska,
Srećko Gajović
Robert Gaspar
Tamas Tabi

Interaction of diphtheria toxin B-subunit with mammalian cells: potential for biomedical application
Kyrylo Manoilov, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine

Nalbuphine: Some Aspects of the Research and Applications
Maksim Logash, Department of normal anatomy. Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Selective inhibition of smooth muscle plasma membrane transport Ca\(^{2+}\), Mg\(^{2+}\)-ATPase by calix[4]arene C-90 and its activation by IPT-35 compound
Olexandr Shrabak, Palladin Institute of Biochemistry National Academy of Sciences of Ukraine, Kyiv; Ukraine

**Consequences of perinatal hypoxia in developing brain: Changes in GABA transporter functioning in cortical, hippocampal and thalamic rat nerve terminals**
Natalia Pozdnyakova/Tatiana Borisova Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine

**Antiplatelet and anti-proliferative action of disintegrin from the venom of Echis multisquamatis**
Volodymyr Chernyshenko, Protein Structure and Functions Department, Palladin Institute of biochemistry NAS of Ukraine

**Glu- and Lys-forms of plasminogen distinctly affect platelet aggregation, secretion and phosphatidylserine exposure**
Yana Roka-Moia/Dmytro Zhermossekov, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

**Ultrastructural changes of the lips' mucous membranes and the mouth corner and the links of their hromomicrocirculatory flow in white rats at the late stages of experimental streptozotocin-induced diabetes**
Julya Hnidyk, Department of Normal Anatomy. Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

**06:15 – 07:15 pm Plenary Session Tea Saloon: Bohdan Malaniak CSMC - RECOOP Young Scientists Research Grants 2016**
4 presentations each for 12 minutes, panel discussion for 12 minutes.

**Chairpersons:**
Sandor G. Vari
Tobor Ertl
Srečko Gajović

**The role of insulin resistance and altered structure of lipid rafts in neurodegenerative disorders**
Fruzsina Bagaméry, Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary

**The role of oxidative stress in development of impaired vascular response in obese pre-diabetic elderly rats of both sexes treated with metformin or liraglutide**
Anita Cosic, Faculty of Medicine Osijek, University Josip Juraj Strossmayer Osijek, Croatia

**The impact of obesity on pregnant rat uterine contractility: crosstalk of adipokines and Wnt proteins**
Judit Hajagos-Tóth, University of Szeged, Faculty of Pharmacy, Department of Pharmacodynamics and Biopharmacy, Szeged, Hungary

**Correlation between placental vascularization indices and sFlt-1/PlGF ratio in preeclampsia screening.**
Ábel Tamás Altorjay, Department of Obstetrics and Gynecology, University of Szeged, Hungary

**07:15 – 08:00 pm Plenary Session Tea Saloon**
- Summary of the CMJ RECOOP Special Issues for Ukraine November 2016
  Srečko Gajović
- Summary of the CMJ RECOOP Special Issues for 2017 April Annual Scientific Review
  Sandor G. Vari
- Review summary Bohdan Malaniak CSMC - RECOOP Young Scientists Research Grants
  Sandor G. Vari
- Summary of Common Mechanism Diseases Breakaway Session
  Sandor G. Vari
- Summary of NanobioTechnology Breakaway Session
  Rostyslav Stoika
- Summary of 7th RECOOP Annual TriNet Project Review Meeting
  Sandor G. Vari

8:00 pm Buffet Dinner Music Saloon

October 9, 2016

Departure
October 8, 2016

8:30 – 10:30 am Plenary Session Tea Saloon

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Sandor G. Vari

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Andrea Gaspar Suranyi

01:00 – 02:00 pm Buffet Lunch – Panorama Room

02:00 – 4:00 pm Plenary Session Tea Saloon
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7 manuscripts each for 12 minutes, panel discussion for 30 minutes.
Chairpersons:
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**The role of insulin resistance and altered structure of lipid rafts in neurodegenerative disorders**
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Ábel Tamás Altorjay, Department of Obstetrics and Gynecology, University of Szeged, Hungary

**05:30 – 06:30 pm Breakaway Sessions**

**Tea Saloon - Common Mechanism Diseases**
**Chairperson:** Sandor G. Vari

**Obesity-induced insulin resistant rats: Interrelation between changes in leptin and cholesterol concentrations and key characteristics of glutamate- and GABA-ergic neurotransmission (project plan)**
Tatiana Borisova Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine

**Coffee Saloon - NanobioTechnology**
**Chairperson:** Rostyslav Stoika

**06:30 – 07:30 pm Plenary Session Tea Saloon**
Summary of the CMJ RECOOP Special Issues for Ukraine November 2016
Srečko Gajović

Summary of the CMJ RECOOP Special Issues for 2017 April Annual Scientific Review
Sandor G. Vari

Review summary Bohdan Malaniak CSMC - RECOOP Young Scientists Research Grants
Sandor G. Vari
Summary of Common Mechanism Diseases Breakaway Session
Sandor G. Vari

Summary of NanobioTechnology Breakaway Session
Rostyslav Stoika

Summary of 7th RECOOP Annual TriNet Project Review Meeting
Sandor G. Vari

8:00 pm Buffet Dinner Music Saloon

**October 9, 2016**

Departure
RECOOP Research Strategy
2016 -2018
Friday
10/07/2016
What I learned from the “HFHS diet induced prediabetic, obese elderly rats treated with Liraglutide (Victoza) and Metformin” study.

Vari SG¹,²
¹International Research and Innovation in Medicine Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA
²RECOOP HST Association, Debrecen, Hungary

**Insulin resistance is a consequence of chronic energy surplus = obesity.**

**Insulin and leptin resistance**
Insulin and leptin resistance as a consequence of HFHS diet. Insulin and leptin signaling interacts. Also, causes of leptin and resistance are not just obesity – it could be any other cause of energy unbalance (immunological causes, chronic stress, endocrine unbalance…).

1. Intake and metabolic balance

**How do cells keep chemical reactions in balance?**
Cells are expert recyclers. They disassemble large molecules into simpler building blocks and then use those building blocks to create the new components they require.

**Catabolic and anabolic pathways**
The breaking down of complex organic molecules occurs via **catabolic pathways** and usually involves the release of energy. Through catabolic pathways, **polymers** such as proteins, nucleic acids, and polysaccharides are reduced to their constituent parts: amino acids, nucleotides, and sugars, respectively. In contrast, the synthesis of new macromolecules occurs via **anabolic pathways** that require energy input (Figure 1).

![Figure 1: Catabolic and anabolic pathways in cell metabolism](image)

Catabolic pathways involve the breakdown of nutrient molecules (Food: A, B, C) into usable forms (building blocks). In this process, energy is either stored in energy molecules for later use, or released as heat. Anabolic pathways then build new molecules out of the products of
Figure 2. Metabolic homeostasis of adipose tissue

One of the main functions of adipocytes is to store free fatty acids (FFAs) during energy surplus and release them during fasting or starvation. However, a number of additional functions have emerged over the past 15 years, including the fact that adipose tissue is a major endocrine organ with a very active secretory pathway. Adipocytes interact with other metabolically active tissues through the release of secretory factors (“adipokines”) as well as pro-inflammatory cytokines that are expressed at low levels in a healthy adipocyte. These factors ensure proper metabolic responses for key target tissues, such as the liver and muscle (where they improve insulin sensitivity), the β cells in the pancreas and cardiac myocytes where they lead to improved survival and function, and the endothelium to ensure proper oxygen and nutrient supply. Fertility is also critically linked to having sufficient amounts of energy in the form of adipose tissue. Moderate levels of leptin effectively communicate with hypothalamic centers in the brain, resulting in proper regulation of food intake and energy expenditure. The endocannabinoid tone is appropriate for the energy reserves present. Adiponectin release from adipocytes is high under these conditions, thereby contributing to elevated insulin sensitivity in a number of different adiponectin targeted tissues, including the liver. Adipsin expression is elevated. Resistin and Sfrp5, as well as pro-inflammatory cytokines (TNFα, IL-6 and MCP-1), are low.
In healthy adipose tissue, there is a higher frequency of macrophages that fall into the general category of alternatively activated, anti-inflammatory “M2-type” macrophages, as well as regulatory T cells (Tregs). Eosinophils are present and help sustain the macrophages in an alternatively activated state. Only low levels of B cells are present in healthy, lean adipose tissue. Low levels of cortisone and 11β HSD1 result in low local cortisol levels. Mitochondrial function is preserved, and there is an adequate supply of oxygen and nutrients for the fat cells due to proper vascularization. Insulin sensitivity is fully preserved due to a low level of local inflammation and low levels of cortisol. As a result, insulin potently stimulates glucose uptake through translocation of the GLUT4 transporter to the plasma membrane. Furthermore, insulin effectively stimulates esterification of FFAs to triglycerides (TGs). On the other hand, responsiveness to sympathetic input is highly preserved, reflecting the high degree of metabolic flexibility that the adipocytes display to adapt to environmental cues. Activation of the β3 adrenergic receptors (not diagrammed) stimulates lipolysis. The FFAs and glycerol released under those conditions can serve as a fuel source for other tissues. However, there is also a relatively high degree of immediate re-esterification of these FFAs and glycerol back into TGs.

Abbreviations used: TNFα: tumor necrosis factor alpha; IL-6: interleukin 6; MCP-1: monocyte chemoattractant protein-1; Rbp4: retinol binding protein 4; Sfrp5: secreted frizzled-related protein 5; 11β HSD1: 11β-hydroxysteroid dehydrogenase type 1.

Metabolic Syndrome ePoster Metabolic Homeostasis - Adipose Tissue

www.nature.com

Philipp E. Scherer, Touchstone Diabetes Center, Department of Internal Medicine, University of Texas Southwestern Medical Center

Cells must balance their catabolic and anabolic pathways

Cells must balance their catabolic and anabolic pathways in order to control their levels of critical metabolites — those molecules created by enzymatic activity — and ensure that sufficient energy is available.

Starvation at cellular level:

If supplies of glucose start to wane, as might happen in the case of starvation, cells will synthesize glucose from other materials or start sending fatty acids into the citric acid cycle to generate ATP. Cells start using fatty acids after depletion of glycogen stores – acute – like in excessive exercise or chronic – prolonged non-feeding – in humans usually longer than 10 hours – so starvation on cellular level, not system level.

Overload at cellular level:

Conversely, in times of plenty, excess glucose is converted into storage forms, such as glycogen, starches, and fats.

Metabolic Pathways

Many of the molecular transformations that occur within cells require multiple steps to accomplish. Recall, for instance, that cells split one glucose molecule into two pyruvate molecules by way of a ten-step process called glycolysis. This coordinated series of chemical reactions is an example of a metabolic pathways in which the product of one reaction becomes the substrate for the next reaction. Consequently, the intermediate products of a metabolic pathway may be short-lived (Figure 3).
Enzymes can be involved at every step in a reaction pathway. At each step, the molecule is transformed into another form, due to the presence of a specific enzyme. Such a reaction pathway can create a new molecule (biosynthesis), or it can break down a molecule (degradation). © 2010 Nature Education All rights reserved. http://www.nature.com/scitable/topicpage/cell-metabolism-14026182

2., Overweight and Obesity

In hypothalamus, leptin activates POMC and CART neurons, and inhibits NPY and AgRP neurons, leading to anorexia. Leptin has effects on feeding behavior, appetite, insulin-glucose axis, and cognitive function (growth, reproduction, sympathetic tone, thermogenesis, stress response, immune function, angiogenesis, bone mass, regeneration…). (A) In leptin sensitive individuals, leptin inhibits insulin biosynthesis and secretion from pancreatic β-cells. **By contrast, insulin stimulates leptin secretion from adipose tissue.** Leptin stimulates hepatic gluconeogenesis and hepatic insulin sensitivity via the hepatic branch of the vagus nerve. Additionally, leptin increases glucose uptake in the skeletal muscle, heart, and brown adipose tissue (BAT) via the sympathetic nervous system. (B) In leptin resistant over-weight individuals, the permeability of the BBB to leptin is decreased in high-fat diet-induced obesity despite the increase in plasma leptin levels. **This impaired transport of leptin across the**
**BBB is one of the causes of leptin resistance.** Insufficiency of leptin signaling in the hypothalamus (induced by hyperleptinemia in obese subjects), causes hyperglycemia and hyperinsulinemia, which lead to diabetes mellitus. Overall just a few nuclei have leptin signaling in the hypothalamus, because others are protected against hyperleptinaemia. There is no explanation for causes of hippocampal resistance or some other brain regions. Peripheral leptin resistance probably develops before or in parallel with central – susceptibility of different tissue is not explored. AgRP, agouti-related protein; ARC, arcuate nucleus; CART, cocaine-and-amphetamine responsive transcript; LHA, lateral hypothalamic area; NPY, neuropeptide Y; POMC, proopiomelanocortin; BBB, blood-brain barrier.


**Phase 1., Change BMI, increase fat in the body!**
The body trying to maximize an efficient energy utilization and therefore exposed to chronic energy surplus, means Obesity!

A., It will increase so called ectopic lipid accumulation skeletal muscle (fat) triggers pathways that impair insulin signaling, leading to reduced muscle glucose uptake! Change in body mass index.

B., Also it will increase the ectopic lipid accumulation in liver and would decrease hepatic glycogen synthesis.

**Phase 2., Muscle insulin resistance**
Outcome of this muscle insulin resistance, due to ectopic lipid (obesity) will lead to liver insulin resistance! Muscle insulin resistance diverts ingested glucose to the liver, and resulting in increased hepatic de novo lipogenesis and hyperlipidemia, fatty liver. Subsequent macrophage infiltration into white adipose tissue (WAT) leads to increased lipolysis, which further increases hepatic triglyceride synthesis and hyperlipidemia due to increased fatty acid esterification.

**Phase 3 Low grade inflammation**
Subsequent macrophage infiltration into white adipose tissue (WAT) leads to increased lipolysis, which further increases hepatic triglyceride synthesis and hyperlipidemia due to increased fatty acid esterification. It results in increased hepatic acetylCoA content, a potent activator of pyruvate carboxylase, and increased glycerol conversion to glucose.

**Phase 4 Substrate regulated processes**
These substrates regulated processes (food intake) are mostly independent of insulin signaling in the liver but are dependent on insulin signaling in white adipose tissue (WAT), which becomes defective or incapacitated by the inflammation in white adipose tissue (subcutaneous and abdominal).

**Treatment, reverse insulin resistance = decrease ectopic lipid**
On a simplified way the therapy shall be that, lose weight, reduce or as we call in scientific way decrease ectopic lipid storage and moderate or reduce macrophage induced white adipose tissue (WAT), and the triggered lipolysis will reverse the roots cause of type 2 diabetes.
3. Regulation by hormones

**Adipose tissue as an endocrine organ.** Leptin is secreted by adipocytes and circulates in the blood in a concentration proportional to fat mass content. In addition to regulation of appetite, thermogenesis and body weight, leptin has multiple other biological actions.

![Adipokines & Insulin Signaling Pathways](https://www.rndsystems.com/pathways/adipokines-insulin-signaling-pathways)

Figure 5. Adipokines & Insulin Signaling


One of the leading risk factors for Type II diabetes is obesity, a condition characterized by an increase in adipose tissue mass, altered adipokines secretion, and chronic or low grade inflammation.

**Adipokines & Insulin Signaling Pathways – missing role of Kisspeptin**

Leptin is a key component of the neuroendocrine circuitry that regulates food intake and energy utilization.
The binding of leptin to its receptor in the hypothalamus inhibits food intake and increases energy expenditure through stimulation of sympathetic nerve activity (SNA). Leptin has multiple other functions, either directly through action in peripheral tissues or through activation of thermogenic and cardiorenal SNA.

Leptin exerts its anorectic effects by modulating the levels of neuropeptides such as:

- **Neuropeptide Y (NPY).**
- **Agouti-related protein (AgRP)**
- **α-Melanocyte-stimulating hormone (α-MSH)**

**Neuropeptide Y (NPY).** In the autonomic system it is produced mainly by neurons of the sympathetic nervous system and serves as a strong vasoconstrictor and also causes growth of fat tissue. (Kuo LE, Kittinska JB, Tilan JU, et al. (July 2007). "Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome". Nat. Med. 13 (7): 803–11. doi:10.1038/nm1611. PMID 17603492.)

**Agouti-related protein (AgRP),** also called agouti-related peptide, is a neuropeptide produced in the brain by the AgRP/NPY neuron. It is synthesized only in Neuropeptide Y (NPY)-containing cell bodies located in the ventromedial part of the arcuate nucleus in the hypothalamus. (Bäckberg M, Madjid N, Ogren SO, Meister B (Jun 2004). "Down-regulated expression of agouti-related protein (AGRP) mRNA in the hypothalamic arcuate nucleus of hyperphagic and obese tub/tub mice". Brain Research. Molecular Brain Research. 125 (1-2): 129–39. doi:10.1016/j.molbrainres.2004.03.012. PMID 15193430.)

AgRP is co-expressed with NPY and acts to increase appetite and decrease metabolism and energy expenditure. It is one of the most potent and long-lasting of appetite stimulators. In humans, the agouti-related peptide is encoded by the AGRP gene. (Shutter JR, Graham M, Kinsey AC, Scully S, Lüthy R, Stark KL "Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice". Genes & Development. 11 (5): 593–602. (Mar 1997). doi:10.1101/gad.11.5.593. PMID 9119224.)


Increased adipocyte number and volume are positively correlated with leptin production, and negatively correlated with production of adiponectin.

Characterizing the mechanisms by which adipokines enhance or interfere with insulin signaling pathways is critical to our understanding of how these factors may contribute to the pathogenesis of metabolic disorders.
Figure 6. Leptin binds to the leptin receptor (LepRb) and activates the receptor-associated kinase JAK2 via transphosphorylation and phosphorylates three tyrosine residues (Y985, Y1077, and Y1138). Leptin-induced mRNA expression of JAK-STAT is inhibited by SOCS3. Insulin and leptin regulate the expression of AgRP and POMC via Foxo1 and signal transducer and activator of transcription factor Stat3. Sirt1 suppresses the Foxo1-dependent expression of the orexigenic neuropeptide AgRP. AgRP, agouti-related protein; FOXO1, Forkhead box O1; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3 kinase; PIP3, phosphatidylinositol 3, 4, 5-triphosphate. (Marie Amitani*, Akihiro Asakawa, Haruka Amitani and Akio Inui The role of leptin in the control of insulin-glucose axis Front. Neurosci., 08 April 2013 | http://dx.doi.org/10.3389/fnins.2013.00051)

Insulin
Insulin, secreted by pancreatic beta cells, is the main regulator of blood glucose levels. It inhibits glucose production in the liver, stimulates glucose uptake in muscle and fat, promotes glycogen and lipid synthesis, and inhibits lipolysis.

Insulin signaling promotes glucose uptake by activating intracellular signaling pathways that promote translocation of the GLUT4 glucose transporter to the plasma membrane. Glucose
transporter type 4, also known as GLUT4, is a protein encoded, in humans, by the GLUT4 gene. GLUT4 is the insulin-regulated glucose transporter found primarily in adipose tissues and striated muscle (skeletal and cardiac).

Additionally, insulin signaling inactivates Glycogen synthase kinase 3 (GSK), which keeps Glycogen Synthase active, thereby promoting storage of glucose as glycogen.

Additionally, insulin signaling inactivates Glycogen synthase kinase 3 (GSK), which keeps Glycogen Synthase active, thereby promoting storage of glucose as glycogen.

A regulation of Gsk3 function by leptin is quite interesting and important also from a side of Wnt signaling due to a fact that Gsk3beta is a key component into regulation of Wnt pathway activation. There is a paper that describes an interplay between Wnt pathway and insulin resistance:

“Wnt activation leads to phosphorylation of key insulin signaling proteins, including GSK3_, in an insulin and IGF-1 receptor-dependent manner. This cross-talk between insulin and Wnt signaling occurs at least in part at the level of the Wnt co-receptor LRP5, which has a profound positive effect on insulin signaling in preadipocytes. This involves a direct interaction between the insulin receptor and LRP5, which occurs in an insulin/Wnt-inducible manner.”

The inducible nature of this interaction is specific to the insulin receptor and is not observed with the IGF-1 receptor. In this context, LRP5 appears to serve as a co-receptor, not only in Wnt signaling, but also in insulin signaling. Notably, the IR/LRP5 interaction could also be a mode of action in the Wnt effect on Akt, ERK1/2, and GSK3_ phosphorylation, which would explain the role of insulin/IGF-1 receptors in this Wnt induced phosphorylation response. Thus, the IR/LRP5 interaction acts as a mechanistic bridge between the two pathways that could play a role in the pathogenesis of insulin resistance and obesity. (Palsgaard J. at al., Cross-talk between Insulin and Wnt Signaling in Preadipocytes Role of WNT co-Receptor low density lipoprotein receptor-Related Protein-5(Lrp5) The Journal Of Biological Chemistry Vol. 287, No. 15, Pp. 12016–12026, April 6, 2012, Doi10.1074/Jbc.M111.337048 https://www.ncbi.nlm.nih.gov/pubmed/22337886)

Insulin signaling can be enhanced or inhibited by adipokines secreted by the adipose tissue!

Obesity is associated with reduced Leptin sensitivity and decreased Adiponectin production, two adipokines that normally enhance insulin sensitivity.

These changes are coupled with an increase in the production of pro-inflammatory cytokines such as TNF-alpha and IL-6, which can negatively affect adipose tissue functions and promote insulin resistance.

**Leptin** - enhances insulin sensitivity
Leptin is an important regulator of energy intake and metabolic rate primarily by acting at hypothalamic nuclei.
This leptin action is through the JAK kinase, STAT3 phosphorylation, and nuclear transcriptional effect
Enhances insulin sensitivity
Increases thermogenesis/energy expenditure
Increases fatty acid oxidation
Activates AMPK in muscle and liver. 5' AMP-activated protein kinase or AMPK or 5' adenosine monophosphate-activated protein kinase is an enzyme that plays a role in cellular energy homeostasis.

**Pro – inflammatory:** leptin increases macrophage and monocyte proliferation rates, thereby increasing the levels of inflammatory cytokines (TNF-α, IL-1, IL-6)

**Adiponectin** – enhances insulin sensitivity
Adiponectin lowers plasma glucose and FFAs.
Increases fatty acid oxidation in muscle & the liver
Decreases hepatic glucose output
Increases glucose uptake in muscle
Activates AMPK alpha in muscle & the liver
Activation of AMPK by adiponectin suppresses endogenous glucose production, concomitantly with inhibition of PEPCK and G6Pase expression.
Phosphoenolpyruvate carboxykinase (PEPCK) is an enzyme in the lyase family used in the metabolic pathway of gluconeogenesis.

**Anti – inflammatory**

**Kisspeptin** - enhances or reduces insulin sensitivity?
Kisspeptin (KISS1) and its receptor (KISS1R) increased in liver and in serum in 2 diabetes mellitus (T2DM)
The kisspeptin impacts leptin in the hypothalamus
Influence hormonal circuit between the liver and the endocrine pancreas in glycemia regulation
Hepatic kisspeptin1 impair insulin secretion
Alterations in the hypothalamic expression of kisspeptin by metabolic leptin
Important regulators of the reproductive function,
Obesity are accompanied by alterations in adipose tissue biology and impaired fertility.
Placental kisspeptin modulates pro-inflammatory cytokine
Markers of insulin resistance in polycystic ovary syndrome

**Pro – inflammatory?**

**TNF – alpha** - inhibits insulin sensitivity
Inhibits Adiponectin production
Interferes with early steps of insulin signaling.
Inhibits Insulin receptor substrate 1 (IRS-1) is a signaling adapter protein, IRS1 tyrosine phosphorylation by promoting its serine phosphorylation.
Activates serine/threonine kinases,
Activates c-Jun N-terminal kinases (JNKs), play a role in T cell differentiation and the cellular apoptosis pathway.
Activates mammalian target of rapamycin complex 1 (mTOR), namely as a nutrient/energy/redox sensor and controller of protein synthesisDecreases Glut4 expression
Increases circulating fatty acid levels
Promotes adipocyte lipolysis – apoptosis!

**IL-6** – inhibits insulin sensitivity
Circulating levels correlate with obesity and insulin resistance
Promotes adipocyte lipolysis - apoptosis!
Increases circulating fatty acid levels

**Pro – inflammatory**
Free Fatty Acids - inhibits insulin sensitivity
Increase insulin-suppressed hepatic glucose production
Decrease glucose uptake
Promote the accumulation of triglycerides & fatty acid – derived metabolites in muscle & the liver

Angiopoietin – like 2 – inhibits insulin sensitivity
Circulating levels correlate with adiposity
Pro - inflammatory

Omentin - inhibits insulin sensitivity
Adipocytokine that is abundantly expressed in visceral fat tis
Biomarker of metabolic risk factors, decreased in obesity
Promotes insulin – mediated glucose transport in adipocytes
Circulating levels inversely correlate to obesity

Resistin - inhibits insulin sensitivity
Produced and released from adipose (similar to a cytokine)
Impairs glucose tolerance
Increases the production of LDL in human liver cells and also
Degrades LDL receptors in the liver, therefore liver is less able to clear 'bad' cholesterol
Accelerates the accumulation of LDL in arteries, increasing the risk of heart disease Inhibits adipocyte differentiation

Retinol – Binding Protein 4 (RBP4) - inhibits insulin sensitivity
Increased levels are associated with insulin resistance, promotes hepatic gluconeogenesis, genetic deletion enhances insulin sensitivity in mice

Intervention
PBEF/Visfatin - systemic NAD biosynthetic enzyme –
Cytokine named PBEF or an insulin-mimetic hormone named visfatin enhances insulin sensitivity
Could affect glucose – stimulated insulin secretion by regulating pancreatic beta cell function

Serpin A12/ Vaspin - enhances insulin sensitivity
The expression of human vaspin (serpinA12) is positively correlated to body mass index and insulin sensitivity
Increases glucose tolerance
Administration improves glucose tolerance and insulin sensitivity in diet – induced obese mice
Anti-inflammatory

4., Metabolic regulations

A cell's daily operations are accomplished through the biochemical reactions that take place within the cell. Reactions are turned on and off or sped up and slowed down according to the cell's immediate needs and overall functions. At any given time, the numerous pathways involved in building up and breaking down cellular components must be monitored and
balanced in a coordinated fashion. To achieve this goal, cells organize reactions into various enzyme-powered pathways.

Not only do cells need to balance catabolic and anabolic pathways, but they must also monitor the needs and surpluses of all their different metabolic pathways. In order to bolster a particular pathway, cells can increase the amount of a necessary (rate-limiting) enzyme or use activators to convert that enzyme into an active conformation. Conversely, to slow down or halt a pathway, cells can decrease the amount of an enzyme or use inhibitors to make the enzyme inactive.

**Enzymes (proteins) regulating biochemical reactions by facilitating the molecular rearrangements**

The cell is able to react to overload of energy utilization (too much to eat) and therefore exposed to chronic energy surplus situation in a mechanical way and solve the problem of the amount of a product. Cells also have an immediate signaling connected to amount of imported glucose – OGlucNac – sugar which is added to different enzymes to modify their function – works in parallel with phosphorilation.

Enzymes are protein catalysts that speed biochemical reactions by facilitating the molecular rearrangements that support cell function. Recall that chemical reactions convert substrates into products, often by attaching chemical groups to or breaking off chemical groups from the substrates.

For example, in the final step of glycolysis, an enzyme called pyruvate kinase transfers a phosphate group from one substrate (phosphoenolpyruvate) to another substrate (ADP), thereby generating pyruvate and ATP as products (Figure 6).

**Figure 7. Glycolysis**

Energy is used to convert glucose to a 6 carbon form. Thereafter, energy is generated to create two molecules of pyruvate.© 2010 Nature Education All rights reserved. http://www.nature.com/scitable/topicpage/cell-metabolism-14026182

**Feedback inhibition**
One of the mechanical ways is the feedback inhibition is one of the most important function of proteins. Due to feedback inhibition, a cell is able to know whether the amount of a product is enough for its subsistence or there is a lack of the product (or there is too much product).

The above mechanism will active and inactive form of the enzyme are altered due to covalent modification of their structures which is catalysed by other enzymes called modifiers are.

Such up- and down-regulation of metabolic pathways is often a response to changes in concentrations of key metabolites in the cell. For example, a cell may take stock of its levels of intermediate metabolites and tune the glycolytic pathway and the synthesis of glucose accordingly. In some instances, the products of a metabolic pathway actually serve as inhibitors of their own synthesis, in a process known as feedback inhibition (Figure 7). For example, the first intermediate in glycolysis, glucose-6-phosphate, inhibits the very enzyme that produces it, hexokinase.

![Figure 8. Feedback inhibition](image)

*Figure 8. Feedback inhibition*

When there is enough product at the end of a reaction pathway (red macromolecule), it can inhibit its own synthesis by interacting with enzymes in the synthesis pathway (red arrow). © 2010 Nature Education All rights reserved.

**Modifiers – one of the covalent modifications is phosphorylation.**

This type of regulation consists of the addition or elimination of some molecules which can be attached to the enzyme protein. The most important groups of modifiers are: phosphate, methyl, uridine, adenine and adenosine diphosphate ribosyl.
Enzymes are proteins that can change shape and therefore become active or inactive. An activator molecule (green pentagon) can bind to an enzyme (light green puzzle shape) and change its overall shape. Note the transformation of the triangular point on the green enzyme into a rounded shape. This transformation enables the enzyme to better bind with its substrate (light pink puzzle piece). In contrast, an inhibitor molecule (pink circle) can prevent the interaction of an enzyme with its substrate and render it inactive.

These groups are joined to or eliminated from the protein by other enzymes. It called covalent modification and the most remarkable covalent modification is phosphorylation.

Enzymes are flexible proteins that change shape when they bind with substrate molecules. In fact, this binding and shape changing ability is how enzymes manage to increase reaction rates. In many cases, enzymes function by bringing two substrates into close proximity and orienting them for easier electron transfer. Shape or conformational changes can also act as an on/off switch.

For example, when inhibitor molecules bind to a site on an enzyme distinct from the substrate site, they can make the enzyme assume an inactive conformation, thereby preventing it from catalyzing a reaction. Conversely, the binding of activator molecules can make an enzyme assume an active conformation, essentially turning it on (Figure 9).

Sometimes, the enzymes involved in a particular metabolic pathway are physically connected, allowing the products of one reaction to be efficiently channeled to the next enzyme in the pathway. For example, pyruvate dehydrogenase is a complex of three different enzymes that catalyze the path from pyruvate (the end product of glycolysis) to acetyl CoA (the first substrate in the citric acid cycle). Within this complex, intermediate products are passed directly from one enzyme to the next.

The management of biochemical reactions with enzymes is an important part of cellular maintenance. Enzymatic activity allows a cell to respond to changing environmental demands and regulate its metabolic pathways, both of which are essential to cell survival.

**Obesity and Pregnancy**

In obesity the delivery is postponed in higher rate, than in normal population. Compared with normal BMI (18.5-24.9), the Odds ratio for postponing EDD increased with increasing BMI; BMI 25-29.9 (OR 0.97); 0.93-1.02), BMI 30-34.9 (OR 1.14), BMI 35-39.9 (OR 1.28), and BMI 40+ (OR 1.73). [https://www.ncbi.nlm.nih.gov/pubmed/27517739](https://www.ncbi.nlm.nih.gov/pubmed/27517739)
Research Strategy

Continuing to explore Common Mechanism of Diseases (CMD).

Focusing on obesity

Obesity epidemic continues around the world and in the U.S., and obesity rates are increasing around the world. The latest estimates are that approximately 34% of adults and 15–20% of children and adolescents in the U.S. are obese. (Nia Mitchell, MD, Vicki Catenacci, MD, Holly R. Wyatt, MD, and James O. Hill, PhD Obesity: Overview of An Epidemic Psychiatr Clin North Am . 2011 December ; 34(4): 717–732. doi:10.1016/j.psc.2011.08.005.)

Health risks associated with obesity:
- Type 2 diabetes, it is estimated that 90% of individuals with type 2 diabetes are obese. It is further estimated that 30% of U.S. adults have prediabetes.
- Non-alcoholic fatty liver disease (NAFLD)
- Hypertension, coronary artery disease,
- Cognitive dysfunction
- Reproductive function, uterus contractility, pre and late term birth
- Obesity during before and during pregnancy will lead to adult onset diseases (metabolic, neurodegenerative, cardiovascular diseases)
- Cancer in many forms (endometrial, esophageal, renal cell, pancreatic, ovarian, breast, colorectal, thyroid, and gallbladder cancers)

Figure 10. Sympathetic nervous system in obesity-related hypertension
We will continue to investigate or implement new research based on the results of the Obese elderly rats with prediabetes treated with Metformin and Liraglutide:

1., Interaction between adipokines (leptin, adiponectin, kisspeptin) and insulin signaling pathways. (Animal study Obese elderly rats with prediabetes treated with Metformin and Liraglutide at Szeged)
The strong relationship between obesity and diabetes is well documented but the underlying mechanisms which link development of obesity to initiation and progression of insulin and leptin resistance are less well understood.

2., Crosstalk between leptin and insulin signaling during the development of obesity (Obese rat study at Szeged, Clinical studies at Semmelweis – living donor, chronic kidney disease – recipient, leg amputation, gut resection). Osijek and Budapest teams will work on leptin and insulin signaling. Budapest team on low grade inflammation.

Crosstalk between leptin and insulin signaling during the development of diet-induced obesity, emphasizing potential interactions between pathways that occur among target sites, and exploring how these interactions may influence the progression of obesity and diabetes. Insulin and leptin receptors are known to share signaling pathways, such as JAK2/STAT-3 (Janus kinase2/signal transduction and activator of transcription3), MAPK (Mitogen activated protein kinase), and PI3K (phosphoinositide 3-kinase). Leptin interact with insulin-like growth factor 1 (IGF-1), insulin, and tumor necrosis factor alpha (TNF-α).

- Leptin signaling can regulate adaptive immunity. It is required for Th17 differentiation through upregulation of transcription of RORγt.
- Leptin signaling can suppress regulatory T cell (Treg) differentiation.
- Inhibition of the leptin receptor blocks macrophage microbicidal and phagocytic functions, as well as the maturation of dendritic cells.
- Leptin can inhibit natural killer (NK) cell activation under certain circumstances, a unique effect not observed in other cell types.

Low grade inflammation, increased lipolysis in adipose tissue (apoptosis). Leptin signaling can regulate innate inflammatory responses, such as cytokine production in macrophages and mast cells, as well as leptin-mediated chemotaxis in granulocytes.

Neuro-inflammation will be investigated by the Zagreb and Osijek Teams

- ICH on neuroinflammation markers, e.g. microglia Iba1 and CD68
- double staining with OBR, stem cell markers, ...

3., Obesity and pregnant uterine contractility: molecular and functional investigation of the roles of leptin, adiponectin and kisspeptin in rats (Pregnant obese rat study at Szeged) Osijek and Budapest teams will work on leptin and insulin signaling. Budapest team on low grade inflammation.

In rats, gestational obesity increases the intensity of spontaneous contractions of the last-day pregnant uterus, but reduces its sensitivity to oxytocin. The impact of obesity and adipokines (leptin, adiponectin, kisspeptin) on the glucose level, contractility of pregnant rat myometrium, and the resistance of the cervix will be investigated. The changes in the expressions of uterine and brain adipokines receptors, plasma adipokines levels, and the grade of prediabetes and low
grade-inflammation in obese pregnant rat and their contribution to uterine contractility will be measured. In a second series of experiments the effect of liraglutide or metformin treatment will be investigated focusing on those parameters that are significantly altered by the obesity during pregnancy.

In obesity the delivery is postponed in higher rate, than in normal population. Compared with normal BMI (18.5-24.9), the Odds ratio for postponing EDD increased with increasing BMI; BMI 25-29.9 (OR 0.97); 0.93-1.02), BMI 30-34.9 (OR 1.14), BMI 35-39.9 (OR 1.28), and BMI 40+ (OR 1.73).


4., Gestational Diabetes, adipokines (leptin, adiponectin and kisspeptin) and low grade inflammation (Clinical study at Szeged: Obese pregnant women)

A., We learned from our previous animal studies the altered adipokines secretion contributes to glucose homeostasis therefore we initiate our hypotheses would be the same mechanism in pregnancy. We investigate both direct and indirect mechanisms: direct mechanisms include regulation of insulin secretion and insulin sensitivity; indirect mechanisms relate to inflammation, regulation of adipogenesis, chemoattraction of immune cells and subsequent effects on glucose metabolism.

Women who develop GDM in pregnancy have a much higher risk of developing T2DM post-partum. Chronic low-grade activation of the immune system (increased plasma inflammatory markers without overt signs of inflammation) play an etiologic role in the development of T2DM. Adipokines have provided novel links between obesity and insulin resistance, and the development of T2DM.

We will investigate the links between GDM is down-regulated by adiponectin and anti-inflammatory cytokines (e.g., IL-4 and IL-10) and up-regulation of leptin and pro-inflammatory cytokines implicated in insulin resistance (e.g., IL-6 and TNF-α).

B., We also investigate the insulin and leptin signaling in placenta, could be compared to those complicated with retarded fetal growth and maternal diabetes in terms of

- placental leptin expression (mRNA, protein),
- leptin receptor expression (protein) and
- cord blood leptin concentrations. In addition, normal first and third trimester placenta were compared in terms of the precise anatomical location of leptin and the leptin receptor.

C., Insulin and leptin, adiponectin and kisspeptin signaling in uterus changes on contractility and could cause pre and late term birth.

Study based on the “Obese rat study” at Szeged

D., Maternal and neonatal leptin and leptin receptor polymorphisms associated with preterm birth.

Leptin (LEP) and leptin receptor (LEPR) are synthesized by the pregnant female and embryo. LEP and LEPR play a role in female reproduction and in pregnancy, and already it is proven
LEP and leptin receptor LEPR linked to preterm birth. Therefore, it would be interesting to investigate maternal LEPR genetic variation in obese pregnant women in correlation of kisspeptin and uterus contractility, ratio of prematurity, length of gestation, length of labor! 

We should look for mechanisms beside polymorphism, like obesity and pregnancy.

The length of study period in obesity in pregnancy or gestational diabetes should be longer, then 9 months (length of human gestation).

- Set inclusion and exclusion criteria before the gestation.
- Analyze the characteristics of population (age, pregestational BMI, social background). Follow up of the newborn and the women (complications, when forms T2DM, etc.)

5. Cardiovascular and renal diseases

Recent advances in our understanding of central nervous system (CNS) signaling pathways that contribute to the aetiology and pathogenesis of obesity-induced hypertension. In GDM the incidence of preeclampsia is higher. In patients, who suffered preeclampsia the risk of cardiovascular diseases is elevated. The brain leptin-melanocortin system in causing increased sympathetic activity in obesity. In addition, we highlight other potential brain mechanisms by which increased weight gain modulates metabolic and cardiovascular functions. Separating the CNS mechanisms responsible for increased sympathetic activation and hypertension and how circulating hormones activate brain signalling pathways to control BP offer potentially important therapeutic targets for obesity and hypertension.

Some special risk factors (e.g. obesity, GDM) may predispose women for cardiovascular/cognitive complications in older ages.
**Figure 11.** Some special risk factors (e.g. obesity, GDM) may predispose women for cardiovascular/cognitive complications in older ages. The studies of Croft P et al. have suggested that, compared with women without cardiovascular events, women with cardiovascular events were more likely to have experienced a preeclampsia/ hypertensive pregnancy disorder/GDM/obesity. Croft O et al.: BMJ.1989;298(6667):165–168. doi: 10.1136/bmj.298.6667.165

We are planning to investigate the special hormonal circumstances in endothelial function, in CNS system in pregnancy. Cell membrane-derived microvesicles are biologically active, themselves stimulating neighboring cells and releasing mitogenic, vasoactive, or inflammatory cytokines that ultimately affect vascular tone, including cerebral blood flow and brain function. Compromises in cerebral blood flow could negatively impact brain structure, and ultimately, cognition. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623746](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3623746)

The studies of Croft P et al. have suggested that, compared with women without cardiovascular events, women with cardiovascular events were more likely to have experienced a preeclampsia/ hypertensive pregnancy disorder/GDM/obesity. Croft O et al.: BMJ.1989;298(6667):165–168. doi: 10.1136/bmj.298.6667.165

**The cardioprotective effect of metformin in model of postmenopausal metabolic syndrome** (animal study in female non-obese ovarietomized rats)

We would like to study metformin, widely-used antihyperglycemic drug with pleiotropic effects in ovarian-hormone-deficient state. We will investigate the effect of metformin on vascular parameters in experimental model of postmenopausal metabolic syndrome. We will use adult female hereditary hypertriglyceridemic rats with insulin resistance and impaired glucose tolerance. In this experimental models will be performed surgical ovariectomy to investigate metabolic changes in tissue associated with vascular impairment in postmenopausal period. The effect of metformin on parameters of vascular health/damage (nitric oxide synthase, methylglyoxal and glyoxalase 1, that is involved in degradation pathway of methylglyoxal) before and after ovariectomy. According to recent studies glyoxalase system and methylglyoxal are strongly associated with pathogenesis of vascular complications. The experimental studies will also focus on genetic expression of connexin 37 in arterial wall and the effect of sex and sex hormone deficiency on its expression. We are now particularly interested in macrophages... - could possibly find some common ground regarding last part of your proposal. Pls look at these papers:


6. Leptin signaling and neural development? (Obese rat study at Szeged)
Obesity during before and during pregnancy will lead to adult onset diseases (metabolic, neurodegenerative, cardiovascular diseases.
Osijek team will investigate foetal and maternal brain – signs of leptin resistance and central inflammation. The main question is the correlation between leptin signalling and neural development.

7. Central insulin resistance: Alzheimer's disease and insulin desensitization in the brain

12. Schematic diagram showing the major factors determining neural control of appetite and regulation of energy balance The brain monitors the internal milieu through a number of hormonal- and neural nutrient-sensing mechanisms and is under constant influence of the environment and lifestyle through the senses and mainly the cognitive and emotional brain.

The two streams of information are integrated to generate adaptive behavioral (food intake) and autonomic/endocrine responses determining nutrient partitioning, energy expenditure, and
overall energy balance. Any of the peripheral and central signaling steps are subject to individual predisposition through either genetic, epigenetic, or non-genetic early life imprinting mechanisms. Huiyuan Zheng, Hans-Rudi Berthoud, Neural Systems Controlling the Drive to Eat: Mind Versus Metabolism DOI: 10.1152/physiol.00047.2007 Published 1 April 2008

Molecular pathways linking insulin resistance and Alzheimer disease

Figure 13. Schematic representation of molecular pathways linking insulin resistance and Alzheimer disease.

Figure 12 shows the schematic representation of molecular pathways linking insulin resistance and Alzheimer disease. Peripheral insulin resistance leads to decrease insulin signaling in CNS, followed by alteration in brain metabolism. Increased Aβ toxicity, Tau hyperphosphorylation, oxidative stress and neuroinflammation are attributed to central insulin resistance, which leads to neurodegeneration.

Chapter 3 By Sung Min Son, Hong Joon Shin and Inhee Mook-Jung Insulin Resistance and Alzheimer’s Disease DOI: 10.5772/23409
Long term research possibilities by the Zagrab and Osijek team

1. Neuroinflammation research with microglia elimination by feeding animals for 3 weeks with PLX3397 in the food. Nature Communications: http://www.nature.com/ncomms/2016/160503/ncomms11499/full/ncomms11499.html

2. Transfer the model to mice (e.g. in my or your laboratory) then we could image neuroinflammation by our unique Tlr2/luc model and use various mouse knock-outs relevant to the topic (e.g. Tlr2 KO).

3. MRI imaging of neuroinflammation - difficult but rewarding.
Keynote Speakers
Friday
10/7/2016
Connection between low grade inflammation and development of leptin and insulin resistance in animals on HFHS diet

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Key words: leptin resistance, insulin resistance, metformin, liraglutide, low grade inflammation

Role of insulin and leptin in maintenance of body weight: Insulin and leptin are the major two hormones regulating energy balance in the body. Both have opposing signalling partners, glucagon or other adiponectins, respectively. While insulin and its partner exert its effect acutely, leptin and other adiponectins signal long term changes in body metabolism. If acute normoglycaemia could not be achieved solely by action of insulin and glucagon additional demands are communicated by stress response axis through corticoids. Similarly, adiponectin actions are further reinforced by changes in reproductive hormones. Such elaborate signalling network finally supports vital functions like growth, reproduction, maintenance of body weight, immune function, stress response, thermogenesis, angiogenesis, bone mass and regeneration.

Obesity is an epidemic: In conditions of economic prosperity and abundance, like in modern world, such elaborate network is designed for robustness and we see steady rise in human longevity through the last century. However, abilities to adjust have tipping point on both ends – starvation and abundance both lead toward degeneration and finally exhaustion of back up mechanisms. Due to clinical implications and epidemic rise in obesity deeper understanding of these modern world phenomenon is required.

Prevention and intervention: From clinical point of view, more beneficial outcome is expected from early intervention, before onset of manifested pathology. In the case of obesity, pathology is extensive, but dominant two are metabolic syndrome and diabetes type 2, both propagated by low grade inflammation. Two currently extensively used anti-diabetic drugs, metformin and liraglutide, are the best candidates for early signs therapy. Aim of our study was to provide data about suitability of such intervention and possible pitfalls. We collected extensive data on system and tissue specific insulin, leptin and other adiponectins signalling as well as status of reproductive hormones and low grade inflammation.

Funding: The study has been funded by RECOOP HST grant, and in part by the Croatian Science Foundation under project number IP-09-2014-2324. Acknowledgement: This study was supported by Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Ethical Committee Approval: Hungarian Ethical Committee for Animal Research: registration number IV/3796/2015.
Project plan for clinical studies on the mechanism of obesity induced insulin resistance
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Key words: project plan

Background: Obesity is an important risk factor for the development of insulin resistance and cardiometabolic disorders. Expansion of adipose tissue especially the abdominal one is accompanied its infiltration by macrophages. Both the invading macrophages and the adipocytes themselves produce a plethora of cytokines and adipokines that in turn induce low grade inflammation. The inflammatory mediators released can interfere with the insulin signaling resulting in the development of insulin resistance. Some obese individuals however do not show the characteristics of insulin resistance thus better understanding the mechanism is needed to identify the critical steps that link obesity to insulin resistance.

Aim: The aim of the present proposal is studying various factors probably involved in the development of insulin resistance in obesity. Their correlations to clinical state would help identifying the most critical steps that would serve as therapeutic target for prevention obesity induced insulin resistance.

Methods: Patients undergoing living donor kidney transplantation, and other abdominal surgeries would be involved. Plasma and visceral and subcutaneous adipose tissue samples would be taken. Obesity and insulin resistance are to be evaluated on the basis of clinical parameters. The following parameters are to be studied and correlated to obesity and insulin resistance. Similar experiments on vascular tissues isolated during amputations and abdominal surgeries could help to link the clinical atherosclerosis to similar biochemical processes as low grade inflammation and altered lipid raft function are also coupled to the development of endothelial dysfunction.

SSAO/VAP-1 is an enzyme and adhesion molecule that participates in the migration of leukocytes to the site of inflammation. Its activity in the adipose tissue is to be determined as it may participate in the macrophage infiltration and consequently in the development of low grade adipose tissue inflammation. Cytokines and adipokines levels are to be determined as they can link inflammation in the adipose tissue to insulin resistance. Fatty acid compounds in the adipose tissue and plasma may participate in the low grade inflammation as polyunsaturated fatty acids serve as substrates for the synthesis of numerous inflammatory mediators. The concentration and ratio of anti-inflammatory omega-3 and pro-inflammatory omega-6 fatty acids are thus to be studied. Glycation pattern of various inflammatory proteins, e.g. immunoglobulins may affect their biological function and/or biological half-life thus its alteration can participate in the low grade inflammatory state of adipose tissue. Lipid rafts are membrane microdomains important in signal transduction by containing high density of receptors and other signaling molecules. Insulin receptor and its substrates are localized primarily to the lipid rafts thus this membrane structure can play important role in the development of insulin resistance. Accumulation of gangliosides, glycosphingolipids in the lipid rafts is reported compromising its function and accompanied by insulin resistance. The composition of lipid rafts is thus to be examined. Its effect to the phosphorylation of insulin receptor and insulin receptor substrate 1 protein are also to be studied as they serve as biochemical basis for the insulin resistance.
Expected outcome: Determination of these parameters and their correlation to obesity and insulin resistance may help identifying the critical link between the two clinical states that can serve as a therapeutic target.

Ethical committee approvals: 21/2015-2972/ 2015/ EKU and SE TUKEB number 258/2015

Funding: The studies planned to fund by RECOOP HST grant

Acknowledgement: This study will be supported by Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC
Obesity and pregnant uterine contractility: molecular and functional investigation of the roles of leptin, adiponectin and kisspeptin in rats (project plan)

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Keywords: pregnancy, obesity, adipokines, rat

Introduction: The increasing prevalence of obesity in young women is a major public health concern. These trends have a major impact on pregnancy outcomes in these women. Obesity appears to be a modifier of human fertility and reproductive outcomes but is not universally associated with adverse outcomes. In rats, gestational obesity increases the intensity of spontaneous contractions of the last-day pregnant uterus, but reduces its sensitivity to oxytocin.

Aims: The impact of obesity and adipokines (leptin, adiponectin, kisspeptin) on the glucose level, contractility of pregnant rat myometrium, and the resistance of the cervix will be investigated. The changes in the expressions of uterine and brain adipokines receptors, plasma adipokines levels, and the grade of prediabetes and low grade-inflammation in obese pregnant rat and their contribution to uterine contractility will be measured. In a second series of experiments the effect of liraglutide or metformin treatment will be investigated focusing on those parameters that are significantly altered by the obesity during pregnancy.

Methods: Rats (n=12) will be weaned from their mothers at 3 weeks of age, and will be fed with high fat & high sugar diet (HFHSD) from 3 weeks of age till their sacrifice as last day pregnant animals. Glucose tolerance test: will be carried out after 6 weeks of HFHSD and at day 20 of pregnancy. Animal will be sacrificed on pregnancy day 22. Organs (fetuses, brain, placentas, gonadal fat, liver) will be weighted. Samples (uterus, brain, fat) will be stored for adipokines (leptin, adiponectin, kisspeptin) and oxytocin receptor analysis, immunohistochemistry and oxidant/antioxidant status analysis. Plasma samples will be measured for insulin, glucose, leptin, adiponectin, kisspeptin, estrogen, progesterone, IL-6, CRP, TNFα, cholesterol and triglyceride levels. Myometrial contractility and cervical resistance studies will be carried out in organ bath.

Conclusions: Our project promises to find answer how the leptin, adiponectin and kisspeptin system alter the function of pregnant uterus and how their roles and actions are altered in obese pregnancy. The detection of plasma levels of these peptides may give a chance to find biomarkers to predict increase or decrease in pregnant uterine contractility. The adipocytes-produced peptides and their receptors may serve as new targets for drug development to reduce the risk of premature birth or prolongation of gestational period.

Ethical Committee Approval: IV./3071/2016.
Funding: The study funded by institutional fund and partially funded by RECOOP HST Association
Acknowledgement: This study supported by Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).
Research activity in 2015-2016 at the Institute of Cell Biology, NAS of Ukraine: achievements, problems and perspectives of development within RECOOP-HST Association
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Keywords: nanomaterials, antioxidants, anticancer drugs, bio-markers, cell signaling

Main Research Achievements (publications in 2015-2016) at the Department of Regulation of Cell Proliferation and Apoptosis are:

1) Novel nanomaterials for: a) drug and gene delivery:
- Application of Cisplatin-C60 Fullerene Complex for Treatment of Tumor Cells in vitro and in vivo // Prylutska et al. Nano Research, 2016 (final revision), IF 8.893
- Complex of C60 fullerene with doxorubicin as a promising agent in antitumor therapy // Prylutska et al. Nanoscale Research Letters. 2015. IF = 2.584

2) Antioxidants in anticancer chemotherapy:
- Antioxidants Selenomethionine and D-pantethine decrease negative side effects of Doxorubicin in NK/Ly lymphoma-bearing mice // Panchuk et al. Croatian Medical Journal, 2016. IF = 1.373

3) Bio-markers of autoimmune diseases:
- Identification of a 48 kDa form of unconventional myosin 1c in blood serum of patients with autoimmune diseases // Myronovskij et al. Biochem. and Biophys. Reports (2016)


4) Novel anticancer drugs: development and bio-evaluation:


5) Cell signaling mechanisms at pathology:


RECOOP-HST Partners: Institute of Macromolecular Chemistry in Prague (actual), Institute of Biochemistry in Ukraine (actual), Lviv National Medical University in Ukraine (actual), Slovak Medical University in Bratislava (potential), Wroclaw Technical University in Poland (potential), Debrecen University in Hungary (potential), University of Zagreb in Croatia (potential), University of J.J. Strossmayer in Croatia (potential), Cedars-Sinai Medical Center in USA (supervising).

Non-RECOOP-HST Partners: Lviv National Polytechnic University in Ukraine (actual), Taras Shevchenko Kyiv National University in Ukraine (actual), Ivan Franko Lviv National University in Ukraine (actual), Institute for Cancer Research at Vienna Medical University in Austria (actual), Ilmenau Technical University in Germany (actual), Institute of Food Biotechnology and Genomics in Ukraine (actual), Institute of Hereditary Pathology in Ukraine (actual), Institute of BioOrganic Chemistry in Belarus (actual), Nencki Institute of Experimental Biology in Poland (actual).

Problems: More RECOOP-HST Partners should be involved in the Research Collaborations.

Acknowledgements: Cedars Sinai Medical Center’s (CSMC) International Research and Innovation in Medicine Program; Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association); Participating Cedars–Sinai Medical Center - RECOOP Research Centers (CRRC). Personal thanks are expressed to Dr. Sandor Vari.
Leptin receptor is present in neural and adrenal gland stem cells, allowing stress influence through leptin signaling

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Key words: neural stem cells, adrenal gland, stem cells, leptin, leptin receptor, stress

Introduction: Stress is an everyday challenge in the modern world affecting multiple organs and functions. Stress alleviates overall potential for regeneration, but molecular mechanism behind is still obscure. Leptin modulates neurogenic niche in hypothalamus which is involved in adjustment of neuronal circuitry controlling energy homeostasis. In this study we hypothesize that leptin also influences the stem cells in adrenal gland and adjusts stress response.

Materials and Methods: 16 male Sprague Dawley rats aged 4-months were used in the study. Animals were divided in control and chronic stress groups. Adrenal glands sections were immunostained using antibodies against leptin receptor (Ob-R), MAP2 neural marker and Ki67 proliferation marker. In order to demonstrate leptin effect on adult stem cells, neural stem cells were isolated from C57Bl/6 mouse embryos, differentiated toward neurons and immunostained using the same markers.

Results: Ob-R was present in the stem cells layer of adrenal gland and in the undifferentiated neural stem cells, but not in differentiated cells. Chronic stress lowered expression of Ob-R in stem cell layer of adrenal gland.

Conclusion: Since leptin regulates glucose metabolism and inhibits stress-induced apoptosis this finding indicates leptin role in stem cells survival.

Source: This study was supported by Cedars Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and by following projects: The Croatian National Foundation (HRZZ–IP–09–2014–2324) awarded to M.H. and by FP7 Glowbrain project (REGPOT–2012–CT2012–316120) awarded to S.G.

Ethical approval: All experiments on animals described in this work received approval from the institutional Ethical Committee and the Croatian Ministry of Agriculture (HR–POK–005). All experiments were carried out in accordance with the EU Directive2010/63/EU on the protection of animals used for scientific purposes.
Triggered liver regeneration in portal vein ligation models

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Key words: portal vein ligation, liver regeneration, atrophy-hypertrophy complex

Portal vein occlusion is a technique used before extended hepatic resections to prevent posthepatectomy liver failure. This therapy redirects portal blood to the liver lobes that will remain after surgery, resulting in hypertrophy (regeneration), while the portal deprived lobes undergo atrophy. In the last decades portal vein occlusion techniques have been adopted by many institutions worldwide as a strategy to increase the pool of patients who candidate to extended liver resections. The aim of this study is to give an overview of the relevant animal models of portal vein occlusion and to discuss the main characteristics of the triggered liver regeneration including the induced hemodynamic, morphologic and functional alterations as well as the underlying molecular mechanisms, which might be of interest in both the laboratory and the clinic.

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EC Permit number: PEI/001/3013-4/2014
The Role of Skeletal Muscle Contractile Duration on Cognitive Functions and Health Outcomes
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Key words: skeletal muscle inactivity, myokines, stress, autonomic unbalance, circadian rhythm, saliva

Introduction. Concept Inactivity Physiology based on “too much sitting is not the same as too little exercise” is relatively new research area, investigated by molecular and physiological methods of investigation (Hamilton M., 2014). Sitting more than 50 min is “second smoking” risk factor for metabolic syndrome, coronary artery diseases, type 2 diabetes, obesity, some cancers, deep venous thrombosis etc. Current data has shown that young people during intensive education in high schools and universities have deterioration in memory and other cognitive dysfunctions which makes impossible to concentrate on work, studying and everyday activities. According to the WHO forecasts the incidence of depression in 2020 will outstrip cardiovascular, cancer and infectious diseases, and more and more young people are suffering from depression. Modern medical students (MS) are a special population group characterized with intensive learning performance, which induced enormous workload, ANS unbalance and FD or GD.

Aim: The aim is to identify risk groups and create non-invasive diagnostic tool for personal control and analyses of health, correction life-style and stress-induced living behavior and provide physiological-based preventive measures for mental health protection of young people.

Methods: 50 MS were interviewed using questionnaire that included stress perception, cognitive function, skeletal muscle contractile duration, circadian rhythmicity, and time duration on IT devices and FD. Anthropometric data, heart rate variability (HRV) and the saliva microcristalization by dehydration of mix saliva were analyzed.

Results: the mean of body mass index was 21.7; overweight was in 4%. Males tend to have slightly lower muscle mass - 39.2% (normal value (N) above 40%) and higher total fat content - 21.4% (N - up to 20.0%). Decreased daily skeletal muscle contractile duration were in 68%. The time of using IT devices >6 hrs/day was observed in 63%. The good sleep quality was in 54% vs poor in 46% participants. 67% of MS confirmed high stress level and in 30% was reflected lower parasympathetic activity by HRV. Type I SC was found in 16%; II and III - 70%; IV - 14% of students and it correlated with ANS dysbalance and FD.

Conclusion. Shortness skeletal muscle contractile duration, circadian dysfunction, and increased stress perception lead to cognitive dysfunction, ANS dysbalance in MS. Maintaining regular skeletal muscle contractile activity and sleep and circadian rhythms is a promising physiological approach to improve outcomes of cognitive function and health in young people. Further studies are required to assess saliva secretotome by “omics” biomarkers of ANS disbalance and fGID.

Study was approved by local bioethics committee 15.02.2016 (N2)
Sex Differences in the Oxidative Stress Level and Antioxidative Enzymes Activity in Obese Pre-Diabetic Elderly Rats Treated with Metformin or Liraglutide

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Key words: antioxidative enzymes activity, TBARS, FRAP, diabetes, obesity

Introduction: Diabetes mellitus and obesity increase the level of oxidative stress. This study aimed to determine serum level of oxidative stress and antioxidative enzymes activity in pre-diabetic obese elderly Sprague-Dawley (SD) rats of both sexes, treated with metformin (increases insulin sensitivity) or liraglutide (glucagon-like peptide-1 receptor agonist who stimulates insulin secretion).

Methods: Male and female SD rats were divided in: 1) control group; 2) group fed high fat-high salt diet (HSHFD) from 20-65 weeks of age; 3) HSHFD+Metformin (50 mg/kg/day s.c.); and 4) HSHFD+Liraglutide (0.3 mg/kg/day s.c). Drugs were given concomitantly to HSHFD from 51-65 weeks of age, after which rats were sacrificed for serum collection and measurement of Ferric reducing ability of plasma (FRAP), Thiobarbituric Acid Reactive Substances (TBARS) and SOD, catalase and glutathione peroxidase activity by spectrophotometry.

Results: No difference in TBARS among female rat groups was observed. FRAP was significantly higher in HFHSD+Liraglutide compared to HFHSD+Metformin and HSHFD groups. Catalase activity was higher in control group compared to other female groups. TBARS and FRAP were similar among male groups. Control males had higher catalase activity compared to other male groups, higher GPx activity compared to HSHFD+metformin and HSHFD+liraglutide and higher SOD activity compared to HSHFD+metformin. Female control rats had significantly higher TBARS compared to male control. Catalase activity was significantly decreased in all diet groups of both sexes compared to respective control groups and decreased in female groups overall, compared to male.

Discussion: Metformin significantly decreased all antioxidative enzymes activity compared to control in male group of rats. Treatment with liraglutide increased FRAP compared to HFHSD+metformin and HSHFD groups in female to control levels, but TBARS was not significantly changed.

Conclusion: There are sex-related differences in the level of oxidative stress and antioxidative enzymes activity. Drugs may modify antioxidative capacity more in female than male. Presence of metformin and liraglutide decrease activity of antioxidative enzymes in male.
Sources of Funding: This work has been supported in part by VIF-MEFOS-15 (Faculty of Medicine Osijek, Croatia) and by RECOOP HST grant.

Ethical Approval: Hungarian Ethical Committee for Animal Research (IV/3796/2015).

Acknowledgement: The study was supported by Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).
The Effect of Environmental Enrichment on Retinal Damage After Prenatal Stress

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Key words: enriched environment, ischemic, retina, prenatal stress

Introduction: Environmental enrichment is the stimulation of the brain by its physical and social surroundings. The beneficial effect of EE has already been proven in several experimental setups. It was shown to be neuroprotective in traumatic, ischemic and toxic brain injuries. The maturation of retinal acuity, which is a sensitive index of retinal circuitry development, is strongly accelerated in enriched environment (EE). Stress during pregnancy may have an impact on the somatic and mental development of the fetus.

The aim of our research was to examine the effect of environmental enrichment on the retinal thickness after ischemic injury in animals suffered prenatal stress.

Methods: Pregnant Wistar rats were exposed to restraint stress for 2 hours daily for 7 days. To induce ischemic injury a group of offspring underwent bilateral common carotid artery occlusion (BCCAO) surgery in 3 months of age. Following, a group of the operated animals were kept in EE for 2 weeks. Altogether we had 8 groups of animals: control (n=11) or prenatally stressed (n=15) and BCCAO operated (n=14) or non-operated (n=12) and animals placed in EE (n=10) or normal cage (n=16). After 2 week retinas were removed and proceeded to routine histology. The thickness of all the retinal layers (outer-inner limiting membrane (OLM-ILM), nuclear layers (INL, ONL), plexiform layers (IPL, OPL)) were measured. The data were analyzed by MANOVA statistical method.

Results: We observed that some retinal layers (OLM-ILM, ONL) were thinner in the prenatally stressed animals than that of the controls when comparing the results of the BCCAO operated animals. EE resulted in thicker OLM-ILM distance and IPL in each observed group.

Discussion and Conclusion: We observed that, the prenatal stress may lead to increased vulnerability and deteriorative consequences in adulthood, such as ischemic injury. While enriched environment can spare the retinal thickness after ischemic damage.

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Ethical Approval: Animal housing, care and application of experimental procedures were in accordance with institutional guidelines under approved protocols (No: BA02/2000-15024/2011, University of Pecs following the European Community Council directive).
Effects of valerian root extract and commercial “valerian-drink” on anxiety-like behavior and oxidative status in rats

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Key words: valerian, anxiety, kidney function, oxidative stress, rat

Introduction: A number of pharmacotherapies have evolved for treatment of affective disorders. These therapies may produce many side effects. Therefore, there is great interest in developing new herbal compounds which are safe and readily available. The aim of the present study was to observe the effect of valerian (Valerian officinalis L.) root extract and commercial beverage containing valerian extract on the anxiety-like behavior and locomotor activity in adult female and male rats and additionally to assess oxidative stress.

Methods: Both female and male Wistar rats were used. Firstly a crossover experiment with extract from Valerian officinalis L. (4mg/kg) was conducted, and in second experiment, animals had continuous free access to commercial drink containing valeriana extract (0.08%). To assess the anxiety-like behavior and locomotor activity, the open field test, Phenotyper, light-dark box test and elevated plus maze test were performed. In addition, the corticosterone concentration, and also the level of oxidative and carbonyl stress biomarkers in plasma were investigated.

Results: Time spent in central zone of open field (F(1,11) = 0.4841, p = 0.5010), time spent in light part of light-dark box (F(1,11) = 3.416, p = 0.0916), and time spent in open arms (F(1,11) = 0.1799, p = 0.6797) in elevated plus maze were not significantly different. In distance moved a significant interaction between gender and treatment (F(1,11) = 5.872, p = 0.0338) was observed. In second experiment, the animals receiving valerian-drink spent more time in light before first entry into the dark part of the light-dark box (F(1,24) = 4.531, p < 0.05). No significant differences were shown in time spent in center zone of open field (F(1,24) = 0.001564, p = 0.9688), time spent in the light zone of the light-dark box (F(1,24) = 0.004244, p = 0.9486) or time spent in open arms (F(1,24) = 1.334, p = 0.2595). There were no significant differences between treatment groups in oxidative (TBARS: F(1,24) = 1.055, p = 0.3147; AOPP: F(1,24) = 0.01874, p = 0.8923) and carbonyl stress biomarkers (AGEs: F(1,24) = 0.4076, p = 0.5292; fructosamine: F(1,24) = 0.2730, p = 0.6061) or in total antioxidant capacity (TAC: F(1,24) = 2.296, p = 0.1427), but significant effect of treatment on ferric reducing activity of plasma was found (FRAP: F(1,24) = 5.133, p < 0.05). In plasma concentration of corticosterone, there were gender differences (F(1,15) = 5.791, p < 0.05). There were no significant differences between experimental and control groups.

Discussion and Conclusion: Valerian extract treatment seemed to be more effective than consumption of valerian drink, and it tended to have anxiolytic effect. Our results suggest that

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the heightened intake of valerian-drink in female rats may cause increase of corticosterone levels and creatinine clearance, but also reduction of antioxidant capacity.

Acknowledgement: Special thanks of authors goes to Good Night Drink, LtD, Ruzova dolina 26, 821 09 Bratislava, Slovakia (www.goodnightdrink.com) for providing their products used in our experiment.

Source of research support: The work was supported by Operational Programme Research and Development in call OPVaV-2012/4.2/08-RO provided by Ministry of Education, Science, Research and Sport of the Slovak Republic, ITMS No: 26240220086.

Ethical Committee approval: The experiment was approved by Ethical committee as No. 09/2012/SKP1012, date of approval 04.06.2012.
**Tissue-Protecting Effect of Antioxidants Selenomethionine and D-Pantethine Towards Doxorubicin Toxicity in B16 Melanoma-Bearing Mice**

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**Aim** was to study therapeutic treatment effects of antioxidants selenomethionine and D-pantethine applied together with doxorubicin (Dx) towards B16 melanoma bearing-mice. Impact of these experimental regimens on tumor volume and median survival time of animals, as well as blood cell profile, status of liver, heart and kidney have been analyzed in comparison with the action of Dx applied alone.

**Methods:** Tumor volume was calculated as ½ Lwidth*Length*Height, measured every 3 days by caliper. Hematological profile was studied by analysis of blood smears under Evolution 300 Trino microscope. Hepatotoxic action of studied drugs was evaluated by measuring activity of ALT/AST enzymes, cardiotoxicity – by LDH assay, and kidney status - by measuring creatinine level.

**Results.** D-pantethine (500 mg/kg) and, to lower extent, selenomethionine (600 µg/kg) caused a partial reduction of negative side effects (leukocytopenia and erythropenia) of Dx (10 mg/kg) action in B16 murine melanoma. They also moderately increased animal survival time from 60 to 70-75+ days and improved the quality of their life. Besides, we observed normalization of cardiac, liver and kidney function in experimental animals treated with novel regimen compared to the action of Dx alone. Thus, we have demonstrated tissue-protective functions of D-pantethine and selenomethionine. These data are also in agreement with our previous results on studying of NK/Ly lymphoma (Panchuk et al, CMJ, 2016). Thus, the antioxidants selenomethionine and D-pantethine possess a positive therapeutic effect towards different experimental tumor models.

**Conclusions:** Antioxidants selenomethionine and D-pantethine partially reversed negative side effects of Dx in B16 melanoma-bearing mice and increased the treatment efficiency of this drug.

**Acknowledgements.** This study was supported by Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars-Sinai Medical Center - RECOOP Research Centers (CRRC).

**Ethics Committee Approval:** Protocol № 2/2016 from 10.05.2016 of the BioEthics Committee of the Institute of Cell Biology, NAS of Ukraine.
Poster Session
Poster Presentations

Friday
10/07/2017
Renal histopathological alterations in diet-induced diabetic, obese elderly rats treated with Liraglutide or Metformin

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Keywords: nephropathy, fibrosis, inflammation, glomerular changes

Introduction: A quarter of the world’s adult population have metabolic syndrome, a cluster of risk factors for development of cardiovascular disease and type 2 diabetes. In turn, longstanding diabetes and cardiovascular diseases, in particular hypertension and atherosclerosis, lead to kidney disease in a considerable number of patients and finally progressing to end-stage kidney disease requiring renal replacement therapy such as dialyses or transplantation.

Methods: We have analyzed kidneys of elderly rats that were either fed with normal diet (healthy control), or (disease control) or high-fat-high-sucrose-diet fed rats treated with liraglutide, an incretin mimic, or metformin, the standard medication in type 2 diabetes patients, both as treatment groups. The kidneys were obtained from the RECCOP consortium members. To reveal the effects of both drugs on the kidneys in this rodent model of metabolic syndrome, we performed a comprehensive, quantitative analysis using histological and immunohistochemical methods, all of which are very well established in our laboratory. All outlined analyses were done in male rats.

Results: Surprisingly, all of the analyzed markers of renal injury, fibrosis and inflammation were similar between healthy and disease control groups. Since at this stage, no prominent and detectable disease specific changes could be observed!

Discussion: In glomeruli, there was no FSGS and difference in the number of glomeruli containing CD44 positive activated parietal epithelial cells, as precursor lesions to FSGS between the treated groups and the controls. Similarly, the amount of fibrosis, quantified in collagen type IV immunohistochemistry, and podocyte damage, using desmin immunohistochemistry, as well as the size of the glomerular tuft, i.e. hypertrophy, which all are typical signs of early obesity and diabetes induced glomerular injury, were not altered significantly between the groups.

In the tubulointerstitium, we could detect significantly more tubular injury in liraglutide treated group compared to the disease control, but not compared to the healthy control. Immune cell infiltration, counting CD44 positive interstitial cells, was significantly increased in the interstitium of the only obese and liraglutide treated animals compared to the control group. Expression of collagen IV was significantly higher in the liraglutide treated group compared to the disease group, while collagen I was not altered. Also no difference was detected in tubular damage using the tubular injury marker lipocalin 2 (LCN2/NGAL).

Conclusion: The presented animal model did not result in any significant obesity or diabetes typical pathological changes in the kidney. Nevertheless, some markers of tubulointerstitial injury, mainly fibrosis and inflammation, showed a negative renal effect of liraglutide treatment.

Next research step: We aim to analyze female rats and are planning to eventually add some additional markers. Correlation with renal function parameters would be helpful.

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All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013).
Analysis of rat plasma samples of elderly rats with HFHS diet and treated with Liraglutide (Victoza) and Metformin.

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Goals: In this study, N-linked glycans from rat plasma and visceral fat samples were investigated by high sensitivity capillary electrophoresis analysis with laser induced fluorescence (CE-LIF) detection. The objective of this work was to reveal possible N-glycosylation changes due to obesity.

Methods: Samples were collected from four groups of rats: 1: control, 2: high fat high sugar diet (HFHSD), 3: HFHSD+metformin, 4: HFHSD+liraglutide
In these groups, there were male and female animals respectively, containing plasma, visceral and subcutaneous adipose tissue samples.
The glycoprotein extraction and release were both performed with radioimmunoprecipitation assay (RIPA) buffer and PNGase F endoglycosidase enzyme.
All samples were solubilized in RIPA buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) with the addition of dithiothreitol (DTT) denaturing agent and incubated at 100°C for 20 min, then 65°C for 60 min. After the solubilization step, iodoacetamide was used for the alkylation. For buffer exchange, 10 kDa spinfilters were used at 5000 × g for 10 min. The N-glycan release was prepared with PNGase F enzyme and took place at 50 °C for 1 hour. The released glycans were labeled with 8-aminopyrene-1,3,6-trisulfonic acid (APTS) fluorescent dye. A PA800 Plus Pharmaceutical Analysis System (SCIEX, Brea,CA) was used to perform all capillary electrophoresis analyses. The separations were monitored by laser induced fluorescence (LIF) detection using a 488 nm solid state laser with a 520 nm emission filter.

For the exoglycosidase digestion, three different types of sialidases were used:
- Sialidase A released α(2-3)-, α(2-6)-, α(2-8)-, and α(2-9)-linked N-acetylneuraminic acid from oligosaccharides and glycoproteins.
- Sialidase N released α(2-3) and α(2-8), -linked N-acetylneuraminic acid.
- Sialidase V released α(2-3,6,8)-linked sialic acid from oligosaccharides, glycoproteins, complex carbohydrates.
Results:

Figure 1: Total N-glycan profile of rat plasma samples (first number means the group, F means female, M means male, followed by the ID number of the animals, then the type of sample - P is plasma, lastly the number of the aliquot)

Figure 2: Male animals

Figure 3: Female animals

APTS labeled maltose was used as internal standard for trace alignment. The upper trace on every figure is a maltooligosaccharide ladder.

No apparent changes were observed between the traces in either panel, suggesting no effect of obesity on the glycosylation of plasma samples. There are very little observable differences between the traces which could have been caused by the sample preparation or variances in the concentration of the samples.

To obtain a better understanding of the changes of glycan structures between the groups, exoglycosidase array based carbohydrate sequencing was applied. Figure 4 shows the traces of the APTS labeled released glycans from the intact rat plasma samples from two male animals as well as after different sialidase treatments. The Sialidase A and V had produced better results, however the samples from different groups showed almost the same profile. There were differences only in the intensities of the electropherograms.
Figure 4: Total released N-glycan profile from two male animals in the control and the HFHSD groups and electropherograms after sialidase treatments. (First number means the group, F means female, M means male, followed by the ID number of the animals, then the type of sample - P is plasma, lastly the number of the aliquot)

Discussion and Conclusion: Next step in these investigations is the analysis of the visceral and subcutan fat tissue samples. One preliminary measurement has already been finished. From every group, visceral fat samples from one female animal were prepared for the measurements with the sample preparation method described above. In all cases APTS labeled maltooligosaccharide ladder and maltose internal standard were used for trace alignment.

Figure 5: Total N-glycan profile of female animals from the four sample groups. (First number means the group, F means female, M means male, followed by the ID number of the animals, then type of the sample - P is plasma, lastly the number of the aliquot)

These results indicate that visceral fat samples show more differences between the sample groups in contrast to plasma samples. There are observable changes in the corresponding peak proportions, which might be informative for the general RECOOP project. The most
spectacular difference compared to the control sample can be observed in the third trace (HFHSD+metformin) in the G4-G5 region.

Further investigations will be necessary for the exact identification of the changes.

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All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013).
Sex-specific development of type II diabetes – model for pathogenesis
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Key words: leptin resistance, insulin resistance, metformin, liraglutide,

Introduction: It is well known that males and females differ significantly regarding the metabolism and energy balance. Due to different reproductive demands, females tend to exhibit higher leptin levels compared to males, and males usually have higher basal insulin levels. It might be that this is the reason why females are more prone to leptin resistance and males are more prone to insulin resistance. We hypothesize that this sex-specific dichotomy might account for different pathogenesis of type II diabetes in males and females.

Methods: Sprague-Dawley rats were divided into four groups based on their diet and treatment: the control group was fed ad libitum on the standard chow, whereas the experimental group was fed ad libitum on high fat & high sugar diet (HFHSD) for 20 weeks. In the fifth week, the experimental group was further divided into three groups, based on the drugs they started receiving: 1) HFHSD rats on no drugs, 2) HFHSD rats on metformin and 3) HFHSD rats on liraglutide. Food consumption and body weight were regularly assessed throughout the experiment. A glucose tolerance test was performed on each animal every month. The animals were eventually sacrificed and tissue samples were collected for additional analyses, including Sudan B staining for quantification of hepatic steatosis, fasting plasma insulin concentration, HOMA-IR index and plasma leptin concentration.

Results: Glucose tolerance test (GTT) revealed that both aging and HFHSD impair glucose homestasis in females in a greater extent than in males. Metformin seems to slightly improve GTT results in both sexes. Liraglutide possibly improves the GTT results in females, but worsens the results in males. HFHSD males exhibit marked fatty metamorphosis in the liver, apparently more profound than females. Metformin does not significantly alter the steatosis either in males or females. Liraglutide seems to alleviate the liver fat burden in females, but this effect is absent in males. Fasting plasma insulin concentration was higher in HFHSD animals compared with the control group, but hyperinsulinaemia did not significantly differ between HFHSD males and females. HOMA-IR index and leptin plasma concentrations were significantly higher in HFHSD rats compared to the controls. However, no differences were found between the HFHSD males and females.

Discussion: Male rats seem to be more prone to hepatosteatosis because of their susceptibility to hyperinsulinaemia. Since insulin promotes glycogen saturation in the liver, all the blood glucose surplus is stored as fat. In addition to lower insulin plasma concentration, females have more body fat than males. Consequently they have a greater fat storage capacity which relieves them of the need to accumulate fat in the visceral organs so extensively. Metformin might not alter liver fat deposition in rats fed on the HFHSD because its inhibition of gluconeogenesis eventually might promote glycogen accumulation and fatty acids synthesis in the liver. Whilst liraglutide possibly slightly improves the GTT results in females, it seems that it worsens the results in male rats because it stimulates insulin secretion, and thus increases the
hyperinsulinaemia in males, which then promotes the liver fat deposition. Fasting insulin concentrations were higher in males in the control group, which was expected. However, later on, after diabetes already developed, both males and females suffer hyperinsulinaemia and diminished insulin responsiveness, as demonstrated by HOMA-IR index. Fasting leptin levels were higher in females in the control group. In HFHSD rats hyperleptinaemia eventually develops in both sexes.

**Conclusion:** Based on our results and other reports, we suggest that prominent sex-specific differences exist regarding type 2 diabetes development. An explanation we propose is that differences in insulin and leptin sensitivity between males and females might be responsible for this. HFHSD burden might cause hyperleptinaemia in females, triggering the leptin resistance. If untreated, hyperleptinaemia would eventually reduce pancreatic insulin release, consequently precipitating diabetes. In males, dietary burden would primarily lead to hyperinsulinaemia and insulin resistance. If untreated, hyperinsulinaemia would advance into hyperleptinaemia and leptin resistance by means of the insulin-mediated stimulation of leptin secretion. Transition from insulin resistance into leptin resistance occurs because high leptin levels inhibit insulin secretion.

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**Ethical Committee Approval:** Hungarian Ethical Committee for Animal Research: registration number IV/3796/2015.
Adiponectin receptors in the brain of rats on HFHS diet

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**Key words**: leptin receptor, insulin receptor, kisspeptin receptor, obesity, diabetes, brain, rat

**Introduction.** Body’s energy balance is precisely controlled by hypothalamic satiety centers which are responsive to signals coming from adiponectins (leptin, kisspeptin etc.) and other hormones involved in maintenance of energy balance (e.g. insulin, glucagon). Chronic overnutrition may disrupt this balance and lead to disease state, such as metabolic disorder or neurodegeneration. The mechanism of disruption of involved pathways is still not clearly explained.

**Methods.** Study included sixty-four male and female 44-weeks-old Sprague-Dawley rats divided in 4 groups: 1. Standard diet (SD), 2. Diet rich in carbohydrates and fat (HFHSD), 3. HFHSD + metformin treatment (50 mg/kg/day), 4. HFHSD + liraglutide treatment (0.3 mg/kg/day). The experiment lasted for 20 weeks. The obesity was induced during first 5 weeks. The treatment started in week 6 and lasted till the end of experiment, when the rats were 64 weeks old. Then the brains were collected and free-floating immunohistochemical staining was performed using antibodies for following receptors; insulin (IR-α), leptin (ObR), insulin-like growth factor 1 (IGF-1Rβ) and kisspeptin (GPR54). The immunopositive cells in hippocampus (HIPP) and hypothalamus (HTH) were counted.

**Results.** Here we present analysis of IR-α and ObR positive cells in HTH satiety regions (arcuate - ARC, lateral hypothalamic - LH, paraventricular - PA, and periventricular nuclei - PE) omitting data about other brain regions. Preliminary data indicate that HFHSD induced downregulation of IR-α and ObR in ARC and PA of female rats what is expected physiological response to HFHSD and a good sign of maintained balance. At the same time, levels of both receptors are equal in males on regular and HFHSD. We supposed that, after so long period of HFHSD, males lost ability to downregulate sensitivity to hyperleptinaemia and hyperinsulinaemia by downregulating of receptor levels in ARC. Metformin and liraglutide treatment of obese females could not bring levels of IR-α to control level (in experimental time), except in LH region. Metformin managed to bring IR-α and ObR levels in ARC of females back to ‘regular chow’ levels and it reflects much lower food consumption while maintaining body weight close to HFHSD controls. Contrary, liraglutide treated females consumed highest amount of food, weighted the least at the end of the study, lost signs of liver stasis, but had the worst glucose tolerance. If compared to HFHSD females, liraglutides females increased ObR in ARC what is sign of developed leptin resistance without development of insulin resistance. Metformin treated males showed similar changes in food consumption and body weight as females. Contrary to females, their IR-α levels in ARC stayed...
at the level of obese animals while ObR went even higher as a sign of leptin resistance. Like liraglutid treated females, males also lowered food consumption and lost some weight, even corrected liver steatosis to some point. Liraglutid significantly downregulated IR-α in ARC and that way lowered insulin sensitivity. At the same time liraglutid treated males increased ObR in ARC what is a sign of leptin resistance.

Discussion and Conclusion. HFHSD is much better endured in females which sustain physiological response even after prolonged period of diet. Males are more prone to development of insulin and leptin resistance in satiety centers. Metformin corrected insulin and leptin resistance in ARC of females on HFHSD, while same treatment was successful in lowering food consumption and body weight, but not leptin resistance in males. Liraglutid corrected insulin resistance in ARC of HFHSD females and even lowered sensitivity to insulin in males, but did not corrected leptin resistance.

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Cellular models of insulin and leptin signaling
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Key words: insulin, leptin, gangliosides, signaling, lipid rafts, glioblastoma cells, myoblasts

Introduction. Two peptide hormones, insulin and leptin, play key metabolic roles by regulating food intake, glucose homeostasis and energy metabolism. Leptin and insulin directly regulate each other - leptin inhibits insulin synthesis and secretion by pancreatic beta cells, and increases insulin hepatic extraction while insulin stimulates leptin synthesis and secretion in the adipose tissue. These two hormones share common effects, their signaling pathways cross talk and are interdependent. Liver, skeletal muscle, adipocytes and astroglia are insulin and leptin dependent and any of these can develop selective or mutually inclusive resistance to mentioned hormones. While insulin and/or leptin resistance in the brain may occur due to damage of brain blood barrier, low grade inflammation or sequestration of their receptors to less favorable lipid raft, peripheral tissue could develop resistance just by low grade inflammation or change in lipid environment. Lipid rafts, a signaling microdomains on cell membranes, regulate cell death and survival with cholesterol and gangliosides as major organizers. We investigated which cell culture models could be used for functional studies of cell selective insulin and leptin resistance and testing hypothesis of ‘unfavorable lipid raft’.

Methods. We tested seven glioblastoma and one myoblast cell lines with antibodies for complex gangliosides (GM1, GD1a, GD1b, GT1b) and GD3, insulin receptor and leptin receptor.

Results. We found one glioblastoma cell line (CRL1620) positive to GT1b, insulin and leptin receptor. Similar result but with less intensity we got with myoblast cell line.

Conclusion. In order to confirm if these cell lines are good models for insulin and/or leptin signaling, we need to perform lipid rafts isolation and confirm co-localization of insulin and leptin receptor with some marker of lipid rafts (such as flotilin).
High fat/sugar diet causes sex specific reactive gliosis in the brain of Sprague Dawley rats

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Introduction: Metabolic changes occurred during after long term high fat/ high sugar diet (HFHSD) can lead to many metabolically related diseases, specifically type 3 diabetes (T3D) lately associated with Alzheimer’s disease. Additional pressure on the body is exerted by aging, mostly on brain’s ability to maintain fully functional internal milieu. Aim of this study was to compare effects of HFHSD on neuroinflammation in Sprague Dawley rats upon treatment with Metformin and Liraglutid in a sex specific manner. Observed parameter was surface area of astroglia, which tends to rise upon astroglia inflammatory response activation.

Methods: Male and female rats were divided in two control groups: one fed with standard laboratory diet (SD) and second fed with standardized HFHSD. Additionally, two experimental groups were fed with HFHSD with introduced therapy – Metformin (MF) (50 mg/kg) or Liraglutide (LG) (0.3 mg/kg). Experiment which included feeding with HFHSD or SD and two types of therapies had lasted for 6 months starting at the age of 9 months. Brains were collected at the age of 15 months and immunostained astroglia marker GFAP. Images were taken using Leica TCSSP8 confocal microscope and analyzed quantified in GIMP software. Statistical analyses were performed in Statistica 12 software.

Results: Inflammation measured with GFAP antibody detection is higher in male and female groups treated with MF and LG if compared with their HFHSD controls (p=0.001 for males MF, p=0.005 for males LG; p=0.002 for females MF, p=0.041 for females LG). In female rat group, astroglia volumes of LG group is significantly higher compared with SD and MF groups (p= 0.01 for SD, p=0.014 for MF), in male rat group that pattern is not observed.

Discussion and Conclusion: The hallmark of reactive CNS gliosis in neurodegeneration and neuroinflammation is characteristic hypertrophy of astrocytes cellular processes. It is also characterized by upregulation of GFAP with formation of the intermediary filament network. The intermediary filament network becomes very prominent particularly in the main processes and the astrocytes soma. Branching out is presented as larger surface area of observed astroglia. Such surface areas of astroglia were observed in all HFHSD groups, and no reduction of total surface of astroglia in groups treated with LG and MF. Females showed steady increasing rate of gliosis in between groups. LG and MF treatments in both male and female groups did not exert neuroprotective effects measured by glial surface area as one of inflammatory markers.

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Ethical Committee Approval: Hungarian Ethical Committee for Animal Research: registration number IV/3796/2015.
Sex differences and sex-hormones role in aortic reactivity to acethylcholine in Sprague-Dawley rats with and without streptozotocin induced diabetes mellitus

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Key words: diabetes mellitus, sex-hormones, aortic reactivity, acetylcholine

Introduction: The effect of sex hormones on vascular reactivity is considered one of the underlying factors contributing to gender differences in cardiovascular functions and diseases. Several reports suggest that hyperglycemia affects male and female vascular beds differently. However, little is known about the interactions between hyperglycemia and sex differences in the vasculature. Present study aimed to investigate if there is a sex-based difference in the aortic relaxation response acetylcholine (ACh) in male and female (non-ovariectomized /ovariectomized (OVX)) Sprague-Dawley rats with and without streptozotocin induced diabetes mellitus (DM).

Methods: Total of 31 rats were divided in 2 groups of male (healthy controls (CTRL, n=5) and streptozotocin-induced diabetic rats (DM, n=5) and 4 groups of female Sprague-Dawley rats: non-ovariectomized healthy rats (non-OVX CONTROLS, n=5), non-ovariectomized diabetic rats (non-OVX DM, n=5), ovariectomized healthy rats (OVX-CONTROLS, n=6) and ovariectomized DM rats (OVX DM, n=5). Bilateral ovariectomy was performed under anesthesia (75 mg/kg ketamine + 2.5 mg/kg midazolam i.p.) at the 5th week of age. DM was induced by streptozotocin (60mg/kg i.p.) at the 6th week of age, induration of 6 weeks before experiments. ACh induced relaxation (AChIR) (10^{-9}-10^{-5} M) was tested in thoracic aortic rings after 5’ noradrenaline (NA) precontraction.

Results: Female controls and non-ovariectomized diabetic rats have better AChIR compared to their male match (male control and male DM respectively). AChIR was similar between DM and control group of rats at both sexes, as well as between male controls and OVX rats. However, AChIR was significantly impaired in OVX DM group compared to OVX CONTROLS.

Discussion: These results suggest that aortic function in female ovariectomized rats exhibits a more prominent impairment compared to non-OVX rats. There is a basic sex difference in relaxation responses.

Conclusion: Study suggests important protective role of female sex hormones and female sex in vascular reactivity.

Acknowledgments: All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local Ethical Committee #2158/61-07-14-124.
Diet induced changes of kisspeptin receptor expression in fatty tissues of elderly rats treated with Liraglutide (Victoza) and Metformin.

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Key words: kisspeptin, elderly rat, adipose tissues, metformin, liraglutide

Introduction: Hypothalamic KiSS-1 gene expression is critical for the maintenance of reproductive function. The kisspeptin gene is expressed in the central nervous system and in several extracranial sites e.g. in the adipose tissues. Recent studies suggest that kisspeptin participates in the regulation of metabolism and signaling, and it has an influence on the energetic and metabolic status in a sexually dimorphic way. In our study we develop an animal model for obesity in aging rats by diet to study the changes of kisspeptin receptor (Kiss1r) expression in the subcutaneous and visceral fatty tissues.

Methods: Male and female rats (44-weeks-old) were put in 4 groups containing 8 male and 8 female rats each: 1. Standard diet (SD); 2. High fat-high sucrose diet (HFHSD); 3. HFHSD+ metformin (50 mg/kg/day); 4. HFHSD+liraglutide (0.3 mg/kg/day) from 51 weeks of age for 14 weeks. Obesity was induced by HFHSD by from 45 weeks of age for 20 weeks. Animals were sacrificed at their 64 weeks of age. The changes in Kiss1r mRNA expression in the visceral and subcutaneous fatty tissues were measured by real-time PCR.

Results: The Kiss1r mRNA expression was the highest in the subcutaneous fatty tissues. The highest expression was measured in the male HFHSD group. The mRNA expressions were similar in the SD and metformin pretreated groups, while it was lower in the liraglutide treated group. In the visceral fatty tissues, the expression of Kiss1r mRNA was significantly lower in the group of HFHSD, metformin and liragluide treated rats as compared with the SD group.

Discussion and Conclusions: The Kiss1r mRNA level was significantly higher in subcutaneous fat. HFHSD was gender-specific effect on the Kiss1r mRNA expression. We have proved that the antidiabetic drugs influence the Kiss1r expression in the adipose tissues of elderly rats.

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EC approval: All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013).
Pregestational BMI: how does it affect the perinatal outcome in diabetic women?

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Keywords: gestational diabetes mellitus, pregestational BMI, perinatal outcome, type-1 diabetes mellitus

Introduction: It has already been known that carbohydrate intolerance develops often during pregnancy. Overweight and obesity represent a major problem in the world, thus the risk for diabetes mellitus increases. In this study we analyzed the influence of pregestational BMI on perinatal outcome in diabetic women.

Methods: During pregnancy care we collected pregestational BMI data of pregnant women. We followed 357 pregnancies, from which 217 women had normal oral glucose tolerance tests, 62 women had gestational diabetes mellitus (GDM) and 78 women suffered from type-1 diabetes mellitus (T1DM). We analyzed the perinatal outcome until the first week following delivery.

Results: The mean value of pregestational BMI was 27.6 in GDM, 29.5 in T1DM and 22.1 in normal cases. The proportion of Caesarean section was 26.3% in normal cases, while in diabetic cases it was significantly higher (75% in GDM and 95% in T1DM group). Perinatal complications were found in 7% of women in normal control group, in 45% in GDM and in 47% in T1DM cases. Necessity for transportation of the newborn to the neonatal intensive care unit was 7.6% in normal cases, 21% (12% intensive care unit, 9% sub intensive care unit) in GDM, 27% (26% intensive care unit, 1% sub intensive care unit) T1DM cases. The mean birth weight was 3351.9 g in normal cases, 2951.1 g in GDM, 3902.1 g in T1DM. On the average, the length of gestation period lasted for 39.1 weeks in normal, 36.7 weeks in GDM and 37.7 weeks in T1DM cases.

Discussion: Pregnancy outcome and perinatal complications show correlation with maternal weight. Carbohydrate intolerance causes gestational diabetes more likely in obesity than in women with normal BMI. Investigation of pregestational BMI is an important factor for follow up and screening of high risk pregnancies.

Conclusion: The pregestational BMI has influence on perinatal outcome.

Ethical Committee approval on September 12, 2011 with registration number 135/2011

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Identification of myoelectric signals of pregnant rat uterus: new method to detect myometrial contraction

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Keywords: contractility, electromyography, uterus, pregnant rat, myometrium

Introduction: The myoelectric processes are crucial for the initiation of myometrial contractions, especially in pregnancy. However, there is no reliable method to detect the electric activity of the myometrium to predict premature contractions or other disorders. Our recent aim was to develop an electromyography method for pregnant rat uterus in vivo and to separate them from the GI tract signals.

Methods: Sprague-Dawley rats (21st; 22nd days of pregnancy) were anaesthetized with ketamine-xylazine and their stomach, small intestine and large intestine were removed from the abdomen. A pair of thread electrodes was inserted into the uterus, while a pair of disk electrodes was placed subcutaneously above the organs. Additionally, we fixed a strain gauge sensor on the surface of the miometrium and caecum for the parallel detection of mechanical contractions. The filtered electric signals were amplified and recorded by an online computer system and analyzed by fast Fourier transformation. The frequency of the electric activity was characterized by cycle per minute (cpm), the magnitude of the activity was described as power spectrum density maximum (PsDmax).

Results: The frequency of the pregnant uterine activity was found at 1-3cpm that falls within the same range than that of caecum. Oxytocin (1µg/kg) increased by 25-50%, while terbutaline (50µg/kg) decreased the PsDmax by 25-40% measured by both electrodes. We found correlation between the alterations of PsDmax values and the strain gauge sensor-detected AUCs. The GI specific compounds (neostigmine-atropine) mainly affected the cecal activity, while myometrium specific drugs (oxytocin-terbutaline) influenced the myometrial signals.

Discussion and Conclusions: Our electromyographic method is able to detect the myoelectric activity that reflects the mechanical contraction. The overlapping myometrial and cecal signals can be distinguished based on their activities. Thus the early signs of contractions can be detected and labor can be predicted in a fast and sensitive way.

Source of research support: This work was supported by project PIAC_13-1-2013-0201, National Research, Development and Innovation Office, Hungarian Government.

Ethical Committee Approval: All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013).

Acknowledgements: The study was supported by Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars-Sinai Medical Center - RECOOP Research Centers (CRRC).
Differential pro-apoptotic effects of novel 4-thiazolidinone derivatives in human glioma cells

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Key words: 4-thiazolidinone, C6 rat glioblastoma cells, human glioblastoma T98G and U251 cells

Introduction. Glioblastoma (GBM) is an invasive brain tumor in adults, and the definite removal of the malignant cells not possible. Temozolomide (TMZ) is the present-day’s gold standard in chemotherapy for GBM patients. Conversely, their median survival time is only 12-15 months due to the developed resistance to TMZ. Recently, the authors demonstrated that novel structurally related synthetic 4-thiazolidinone derivatives ID 3288, ID 3833, ID 3882 effectively killed rat C6 glioma cells [1]. In present study, human glioma T98G and U251 cells were treated with these compounds and compared to Doxorubicin.

Methods and Materials. ID 3288, ID 3833, and ID 3882 compounds were synthesized by the team of Roman Lesyk at LNMU [2]. Human T98G and U251 glioblastoma cells were treated with these compounds parallel with TMZ, and their biological effects were monitored by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue (MTT) assay and Western-blot analysis of the apoptosis-related proteins. Expression of the anti-apoptotic proteins and degradation of DNA reparation enzyme were monitored. Annexin V test and flow cytometry analysis for cell cycling carried out for human glioma cell lines.

Results and Discussion. Half maximal inhibitory concentration (IC₅₀) ranking of a decrease in value of vitality and assessing cell metabolic activity with MTT assay of human glioblastoma T98G and U251 cell lines are the following: ID3882<Dox<ID3288<ID3833, comparing with ID3882<ID3833<ID3288~Dox counted earlier for C6 rat glioblastoma cells [1]. Rating of compounds based on an increase in number of apoptotic human glioma is: ID3882<ID3288<ID3833<Dox. This ranking is close to standing detected for amount of cleaved caspase-3 (biochemical marker of apoptosis): ID3882<ID3288<ID3833>Dox. Comparing the IC₅₀ for studied 4-thiazolidinone derivatives in human glioblastoma cells with IC₅₀ detected for TMZ [3], one can state higher cytotoxicity of these derivatives, especially ID 3833 and ID 3288.

Conclusions. (1) 4-thiazolidinone derivatives kill both rat and human glioblastoma cells; ID3833 and ID3288 are the most active, while ID3882 is cytotoxic only in high doses. (2) cytotoxic action (IC₅₀) of ID3833 and ID3288 for human glioma cells is more pronounced than the action of TMZ. (3) The apoptosis mechanisms (increase in number of pre-G1 and AnnexinV-positive/Propidium iodide negative cells, expression of biochemical markers of apoptosis, such as cleaved caspase 3) were demonstrated in the toxic action of ID3833 and ID3288 towards glioma cells.

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Neuroprotective effects of 4-thiazolidinone derivatives in rat model with MPTP-induced Parkinsonism

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Key words: experimental parkinsonism, 4-thiazolidinones, oxidative stress

Introduction: Parkinson’s disease is one of the most common progressive neurologic diseases with a high prevalence in elderly population. Dopamine precursor L-dopa in combination with carbidopa is the optimal substitution therapy. The aim of the present study was to identify among thiazolidin derivatives means with antiparkinsonian activity on the base of pharmacological screening and on the model of MPTP-induced parkinsonism.

Methods: On the base of pharmacological studies, grounded by virtual screening, a group of non-toxic lead compounds which are characterized by high antiparkinsonian properties was chosen. The experiment has been carried out on white rats with the weight of 28—320 g. Five groups of animals underwent the experiment: control group, MPTP (MPTP 30 mg/kg), L-dopa (MPTP 30 mg/kg + L-dopa 50 mg/kg) as positive control, 3 groups of animals with MPTP-induced parkinsonism (MPTP 30 mg/kg) treated by experimental substances in the doses of 1/10 LD50 during 10 days.

Results: It was established that studied 4-thiazolidinone derivatives promote the decrease of lipid peroxidation products (DC and MDA) and activate the enzymes of antioxidant protection (SOD and catalase) in brain tissues at the same time. It was revealed that in experimental conditions application of studied compounds increase the activity of thiosulphatdithiolsulphattransferase and cystationin-β-synthase that leads to increased level of hydrogen sulphide.

Discussion: Increased oxidative stress may play role in development of Parkinson’s disease. Present treatment mainly ameliorates the symptoms, but doesn’t retard the neuron degeneration, therefore new therapeutic means are needed to delay the neurodegenerative processes.

Conclusion: The data obtained let us to enhance experimental background of Parkinson’s disease treatment.

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Acknowledgements: Ihor Nektegayev for technical support
Ethical Committee Approval: 14.03.2016 N3 Danylo Halytsky Lviv National Medical University
Blood Serum 48 kDa Form of the Unconventional Myosin 1c as a New Potential Biomarker for Early Stages of Multiple Sclerosis
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Key words: unconventional myosin 1c, blood serum biomarker, multiple sclerosis, clinical characteristics

Introduction: Monitoring of multiple sclerosis (MS) requires additional molecular markers. Recently, we used original TCA-precipitation/extraction approach in a combination with MALDI TOF/TOF mass-spectrometry and identified earlier unknown 48 kDa form of the unconventional myosin IC isoform b (Myo1C) in blood serum of the MS patients (S. Myronovskij et al., Biochem. Biophys. Reports, 2016). The aim of present work was to estimate a correlation value between the presence of Myo1c in blood serum of the MS patients and clinical characteristics of this disease.

Methods: Serum was obtained from the peripheral blood of 61 MS patients (diagnosed according to McDonald criteria for MS) and 20 healthy volunteers. Unconventional form of the myosin 1c (Myo1c) was prepared, as described (Myronovskij et al., 2016). To identify Myo1c in a pool of proteins extracted by TCA from patients’ blood serum, polyclonal rabbit anti-Myo1c antibody (1:1,000) was used. For statistical analysis, Spearman’s rank correlation was used.

Results and Discussion: Correlations between the presence of Myo1c in blood serum of the MS patients and the term of the disease onset, primary symptoms, disease types, duration, number of relapses, disability status, as well as its dependence on patients’ age, sex, profession, and family history, have been analyzed. Statistically significant correlation (p<0.05) was identified between early age of the MS onset, mild severity of disease, and its debut as a retrobulbar neuritis. In patients’ with high level of the Myo1c, disease duration was significantly shorter (3.6 ± 1.1 years) compared to patients’ without Myo1c in blood serum (8.1 ± 1.2 years). High level of the Myo1c was reliably more often found in patients with a remitting-relapsing type of the MS (p<0.01), while in patients with a secondary progressive type of the MS, the Myo1c protein was not detected in blood serum (p<0.05).

Conclusion: The amount of the Myo1c in blood serum of the MS patients is associated with early stages of this disease, when its diagnostics is the most complicated.

Funding source: This work was partially supported by West-Ukrainian Biomedical Research Center.

BioEthics. Bioethical protocol N2 dated by February 15, 2016 was approved by the Bioethics Board of Danylo Haltsky Lviv National Medical University.

Acknowledgements. This study was also supported by Cedars Sinai Medical Center’s International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association), and the participating Cedars-Sinai Medical Center - RECOOP Research Centers (CRRC).
Poster presentations
Saturday Morning
10/08/2016
Soluble fms-like Tyrosin Kinase-1 (sFLT-1) to Placental Growth Factor (PIGF) Ratio as Possible Indicator for Severity of Preeclampsia

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Key words: preeclampsia, soluble fms-like tyrosin kinase 1, placental growth factor, ratio

Objective: Main purpose of this study was a clinical validation of prenatal determination of soluble fms-like tyrosin kinase 1 (sFLT-1) to placental growth factor (PIGF) ratio.

Methods: Maternal second trimester serum samples at 24 ¹/₇-28 ⁰/₇ weeks of gestation and 28 ¹/₇-32 ⁰/₇ weeks of gestation were collected according to a standard operating procedure and analyzed retrospectively. Levels of antiangiogenic sFLT-1 and proangiogenic PIGF were determined by electrochemiluminescence immunoassay platform and were used to calculate the sFLT-1/PIGF ratio in 35 pregnant women. Totally 12 patients with preeclampsia (PE) and 23 women with normal pregnancy outcomes (CTR) were included in this study. PE group was subdivided into PE group with elevated sFLT-1/PIGF ratio (PE+) and PE sFLT/PIGF ratio (PE-) with cut-off ratio value of 35.

Results: Patient in PE+ group had significantly higher incidence of intrauterine growth restriction (IUGR), smaller GA at the time of delivery and lower infant birthweight compared to other two groups. We noticed negative correlation between sFLT-1/PIGF ratio and GA at the time of delivery and between sFLT-1/PIGF ratio and birthweight at the time of delivery. The value of sFLT-1/PIGF ratio was significantly elevated in PE+ group at both sample collections compared to other groups. Between these three groups there was no difference of age, body mass index (BMI) or smoking habits. All of PE group pregnancies were terminated by caesarian section compared to 25% in CTR group.

Conclusions: Authors found that the sFLT-1/PIGF ratio could be used as an indicator for the development and estimation of the severity of PE and provide objective evidence for the management of patients who will develop preeclampsia.

Funding: Electrochemiluminescence immunoassay platform was provided by Roche Diagnostic.

Ethical Committee Approval: Ethical Committee of University Hospital Osijek registration number 25-1:227-4/2011
B16F10 Murine Melanoma as A Promising Model for Simultaneous Evaluation of Therapeutic Efficiency and Side Effects of Novel Anticancer Drugs

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Key words: murine melanoma, anticancer, cytotoxicity, landomycin A

Aim was to develop a novel efficient test system for evaluation of therapeutic activity and potential side effects of experimental anticancer drugs based on B16F10 murine melanoma using modern biochemical assays. The developed test model was used for pre-clinical studies of novel anticancer antibiotic landomycin A (LA) compared to gold chemotherapy standard doxorubicin (Dx) which is widely used for treatment of solid tumors.

Methods. Cytotoxicity of LA and Dx was measured using Trypan Blue exclusion assay and MTT assay. Hematological parameters of experimental animals were analyzed by counting of erythrocytes and leukocytes amount as well as leukogram. Tumor inoculation was done by a subcutaneous injection of B16F10 cells suspension in an amount of 1 mln per one animal. The length, width, and height of tumors were measured every three days with calipers. Tumor volume was calculated as: Vol = ½ L_width*L_length*L_height. Hepatotoxicity of studied drugs was evaluated by measuring activity of ALT/AST enzymes.

Results. LA possessed a significant dose-dependent cytotoxic activity against B16F10 melanoma cells in vitro, and its LC50 = 2 μM was 5 times lower than that of the Dx. LA at concentration of 10 mg/kg body weight did not cause pathological changes, mortality or general toxic symptoms in intact mice of C57black/6 line. Upon subcutaneous injection, B16F10 formed large, aggressive tumors in C57black/6 mice in 14 to 21 days. To determine whether LA limits melanoma growth in vivo, the intraperitoneal injection of LA (cumulative dose 10 mg/kg) was carried out in B16F10 melanoma-bearing mice. As a result, the mean of tumor volume was reduced significantly (742 mm3) compared with that in control group (3,126 mm3) on 22th day of the experiment. Group of mice treated with Dx also did show a decrease in tumor volume (1,878 mm3 comparing with 3,126 mm3 in control), however the tumor size in Dx-treated mice was 153% larger compared to LA in the same concentration. It was shown that Dx increased in ALT/AST ratio (De Ritis ratio) from 1.3±0.1 (normal value in healthy mice) to 1.8±0.1 indicating hepato- and cardiotoxicity induced under treatment. This index also remained within normal limits (1.2±0.2) in animals with B16F10 melanoma without treatment. In case of LA application, De Ritis ratio increased insignificantly compared to control groups and showed 1.5±0.1.

Conclusions. Landomycin A effectively inhibited the growth of B16F10 melanoma without a marked myelosuppressive effects or cardio- and hepatotoxicity that are typical for Dx action. These results indicate on a great promise of application of landomycin A in chemotherapy of malignant tumors. The developed B16 melanoma test system is planned to be further enhanced with specific nephro- and cardiotoxicity tests to ensure all possible side effects of experimental drugs will be monitored in a single experiment.

Acknowledgement: Support of RECOOP HST Association and the Cedars-Sinai Medical Center is acknowledged. We thank Prof. W. Berger from the Institute of Cancer Research at Medical University of Vienna for providing us B16F10 melanoma cell line.

Mebrofenin transport test for the quantification of hepatic function following portal vein ligation

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Key words: portal vein ligation, liver regeneration, hepatobiliary scintigraphy

Introduction: Selective portal vein ligation (PVL) induced atrophy of portal deprived liver lobes and compensatory hypertrophy of contralateral liver segments. Although, the PVL induced morphological alterations is well-documented, the parallel changes in liver function is still the subject of controversy. Therefore, the aim of the present study was the evaluation of temporal characteristics of hepatic function following PVL.

Methods: In male Wistar rats (n=36) 80% PVL was performed. Indocyanine green (ICG) clearance, liver weight and histopathological analysis (HE; Ki-67) was determined preoperatively and 24h/48h/72h/168 hours after surgery (n=6 each). Different animals (n=6) were subjected to ⁹⁹mTc-mebrofenin hepatobiliary scintigraphy (HBS) to quantify global (uptake: B₁/₂, excretion: D₁/₂) and regional (Tmax, T₁/₂) hepatic function.

Results: Ligated (L) lobes underwent atrophy, while non-ligated (NL) lobes hypertrophysized. ICG-clearance (PDR, RT15) and HBS (B₁/₂, D₁/₂) both displayed transitional suppression of global hepatic function, which recovered by the 168th. PVL decreased regional mebrofenin excretion in both lobes, however, after 72h, NL lobes gradually retained their original values, ultimately exceeding excretion rates of L lobes by the 168th (CpsNL/L₀h=1.3; CpsNL/L₁₆₈h=3.2).

Discussion and Conclusion: Our results indicate that the PVL induced regenerative process is initially promoted at the expense of liver function. After the peak of cell division, however functional capacity of the liver recover, during which ⁹⁹mTc-mebrofenin HBS verified a shift in hepatic function towards NL lobes, which is in accordance with ICG-clearance and the observed morphological changes.

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Modulatory Effect of γ-Fe₂O₃ Nanoparticles, Functionalized with Ascorbic Acid, On Antitumor Activity of Doxorubicin In Vitro

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Key words: cytotoxic activity, nanoparticles, doxorubicin, ascorbic acid

Aim of the current study was to evaluate cytotoxic activity of novel multifunctional γ-Fe₂O₃ nanoparticles, coated with SiO₂, glucose and functionalized by ascorbic acid, towards tumor cell lines in vitro. Additionally, their impact on cytotoxic activity of well-known anticancer drug doxorubicin (Dx) was investigated in vitro.

Methods: Human carcinoma and leukemia cell lines (Jurkat, HL-60/wt, HL-60/vinc, SW 1573/wt and MCF-7) were treated with γ-Fe₂O₃ nanoparticles and their modified forms. Short-term (24 hours) cytotoxic effect of anticancer drugs was studied under the Evolution 300 Trino microscope (Delta Optical, Mińsk Mazowiecki, Poland) after cell staining with Trypan blue (0.1%). For estimating the impact of the studied nanoparticles on cytotoxic activity of Dx, semi-lethal doses of this drug causing death of 50% of malignant cell were used in combination with non-toxic doses of the nanoparticles. All experiments were repeated 3 times.

Results. All samples of γ-Fe₂O₃ nanoparticles and their modifications used in 50 μg/ml and 250 μg/ml doses were found to be non-toxic toward the studied cell lines, while in 500 μg/ml dose they demonstrated moderate toxic effect towards human cancer cell lines, decreasing the percentage of alive cells even by 10-20% compared to the control. All γ-Fe₂O₃ nanoparticles and their modifications used in physiologically harmless concentrations (50 μg and 250 μg) enhanced cytotoxic action of doxorubicin toward human leukemia cells of HL-60/wt and Jurkat lines, decreasing the percentage of alive cells by 5-28% depending on modification of nanoparticle. The highest enhancement of cytotoxic activity of Dx was observed for nanoparticles, coated with ascorbic acid, while combination of the same dose of ascorbic acid and Dx in free form hasn’t shown any cumulative effect. Thus, joint application of Dx and γ-Fe₂O₃ nanoparticles, functionalized with ascorbic acid, may be a promising approach in treatment of malignant tumors.

Conclusions: Novel γ-Fe₂O₃ nanoparticles, coated with ascorbic acid, enhance cytotoxic activity of doxorubicin in vitro towards various human tumor cells.

Acknowledgement: Support of RECOOP HST Association and the Cedars-Sinai Medical Center is acknowledged.

Ethics Committee Approval: Protocol № 2/2016 from 10.05.2016 of the BioEthics committee of the Institute of Cell Biology, NAS of Ukraine.)
HPLC analyses of iron nanoparticles planned to conjugate with anticancer drugs, but it was not proven

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Keywords: HPLC analyses, iron nanoparticles, conjugation, anticancer drugs

Introduction: Obtaining nanoparticles requires extensive characterization and determination of the drug content within the nanoparticles. This parameter must be properly verified because the drug must be efficiently loaded into the nanoparticles to reach its therapeutic goal. Therefore, a suitable quantification method is required, such as UV-Vis spectroscopy or HPLC-UV/Vis methods. In this work, a reverse-phase HPLC-PDA method was developed for the rapid, simple, and optimized determination of the encapsulation efficiency of N,N-dimethylacrylamide/acrylic acid coated magnetic (Fe₂O₃) nanoparticles containing 4-thiazolidinone derivatives (compound 3288, 3882 and 3833). The novel synthetic heterocyclic 4-thiazolidinone derivatives have promising antitumor activities.

Methods: The analysis was conducted using a Shimadzu HPLC system and a C8 analytical column (Kinetex). Chromatographic analyses were performed with a mobile phase consisting of a methanol and water mixture (80:20, v/v) pumped at a flow rate of 1.0 mL/min. The detection wavelength was 245 nm.

Results: The complexes of the 4-thiazolidinone derivate were not soluble in water, only in DMSO. Reference standards of conjugates were not available. An HPLC method for the determination 4-thiazolidinone derivatives was developed, but the existence of conjugation with iron nanoparticles was not proven by HPLC method.

Conclusions: After solution of the solubility problems of the 4-thiazolidinone derivatives and verification of the conjugation reaction between the analytes and nanoparticles with other methods (for example size exclusion chromatography, dynamic light scattering or zeta potential measurements), the HPLC method will be suitable for the determination of encapsulation efficiency and loading capacity of the conjugates. But without reference standard compounds, HPLC is not the most appropriate method for quality control.

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Effectivity of health screening in Hungarian General Practitioners’ Communities: role of medical auxiliaries in prevention

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Key words: Screening, General Practitioner, Praxis Community, Cost Analysis

Introduction: The increase in aging population and healthcare costs will lead to the appreciation of prevention. Since the health-awareness level of society is low, prevention is would primarily be the task of General Practitioners. However, in Hungary 5% of the GP practices permanently vacant, thus the additional burden of prevention is unacceptable to the GPs. Solution can provide by a new form of supply, a community practice in which the participating GPs can apply shared medical auxiliaries for prevention. The Primary Care Developmental Model Program consisting of 4 praxis communities (24 GP practices altogether) operating in 16 deprived settlements aimed to increase the participation of the insured citizens in prevention improving their health status without overloading the GPs. Our paper aims to investigate the operational- and cost effectiveness of the program alongside the main epidemiologic findings.

Methods: Health screening-related data was gathered from the praxis community’s financial data was gathered from the head office of the project. Financial cost analysis is based on international health care cost analysis recommendations. Epidemiologic data was analyzed with SPSS statistics 22 software package.

Results: On the three-year horizon of the project, praxis communities could accomplish the 69% of health screening of the whole insured population (22,400 citizens out of 32,470). The unit cost of screen one insured person was HUF 11,572 approximately 37 Euros.

Discussion: The health screening ratio of praxis communities is four time higher than a GP accomplish without medical auxiliaries. Prevention cost for a citizen is, which is only 5% of the annual expenditure for the diabetic patient under 40.

Conclusion: Our study demonstrated that a GP Community has essential role in prevention, because with the involvement of shared medical auxiliaries, even the overburdened GPs can effectively support the health screening.

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Ethical approval: No ethical committee approval needed
**4-Thiazolidinone-based Derivatives Rescue TNFα-Inhibited Osteogenesis in Mouse Mesenchymal Precursor Cells**

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**Key words:** 4-thiazolidinones, inflammation, bone morphogenetic protein, mesenchymal precursor cells.

**Introduction.** Inflammation is a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off both the injurious agent and the damaged tissue. Rheumatoid arthritis (RA) is a severe autoimmune inflammatory disorder whose etiology remains unknown. It has been demonstrated that tumor necrosis factor α (TNFα) plays a crucial role in tissue inflammation including that of RA pathophysiology. On the other hand, the bone morphogenetic protein (BMP) and Wnt regulatory pathways are key players in signaling mechanisms that induce and support cartilage and bone formation and maintenance. A negative interaction between pro-inflammatory signals and skeletogenic pathways occurs at the sites of inflammation.

**Aim.** To evaluate anti-inflammatory activity of novel 4-thiazolidinone-based derivatives towards TNFα–induced inflammatory processes during osteoblast differentiation in mouse mesenchymal precursor cells.

**Methods.** The multifunctional heterocyclic 4-thiazolidinone-based derivatives (compounds Les4368, Les4370, Les3882, and Les3288) were synthesized at the Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University. We performed in vitro evaluation of functional effect of 4-thiazolidinone derivatives (compounds Les4368, Les4370, Les3882, and Les3288) at different doses (1 μM, 0.3 μM and 0.1 μM) on TNFα induced inhibition of bone formation by mouse mesenchymal precursor cells of C2C12. These cells were induced to differentiate into osteoblasts by different BMPs. Western blot analysis was used to elucidate a mechanism of anti-inflammatory effects.

**Results and Discussion.** We found that treatment of C2C12 cells with TNFα completely inhibited the myoblast differentiation, as well as strongly inhibited BMP-induced osteogenesis. Treatment of these cells with 4-thiazolidinone derivatives (Les4368, Les4370, Les3882, and Les3288) allowed in case of Les4368 and Les3882 to rescue the osteogenic differentiation from negative effect of TNFα, and even to convert it from inhibitor of osteogenesis into its potentiator compared with control. Possible involvement of NF-κB modulation as a key mechanism mediating anti-inflammatory effects was validated by immunoblot assays.

**Conclusion.** Novel 4-thiazolidinone derivatives, Les4368 and Les3882, rescue osteogenesis from negative control of inflammation. The best effect was shown by compound Les3882 that stimulated osteoblast differentiation at low dose (0.1 μM), presumably via modulation of NF-κB pathway.

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To get or not to get supplementation during malignant diseases

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4Szent István Egyetem Kertészettudományi Kar, Genetika és Növénynemesítés Tanszék
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Keywords: cancer, blood, optimal-supplementation,cancer-diet

Introduction: Colon cancer is the second most common malignancy in both men and women. In Hungary, according to the most recent National Cancer Registry, 5626 men and 4781 women cases were registered in 2012 (last available data). Nowadays beside the traditional chemotherapy several alternative therapies are used to enhance the antitumor effect of intravenous therapy. Majority of patients use dietary supplements or hold special diets during their illness. The useful supplements are based on the beneficial effects of antioxidants. We are about to find objective measuring methods to follow the redox homeostasis changes in patients.

Methods: Peripherial blood samples were collected from patients suffering from solid tumors before the first cycle of chemotherapy and were also taken before the third cycle. Each patient’s hematological parameters were specified and redox parameters were measured (inducible chemiluminescence, hydrogen donor activity and free sulfhydryl concentration).

Results: We found no remarkable differences between the redox parameters of the first and second blood samples. There were mostly moderate differences between the compared samples in hematologic parameters. Red blood cell count has been decreased only 2.9%, hemoglobin content has been decreased 1.3%, and platelet count has been decreased 11.5%, but white blood cell count decreased 26.5% which seemed to be a significant difference (p<0.05). Despite of our expectation, the antioxidant defense in most cases was even better before the third cycle of the chemotherapy. Inducible chemiluminescence in red blood cells elevated 67.1% however in blood plasma it decreased 31%, but according to the high standard deviations these results were not significant. Hydrogen donor activity decreased 2.9% and free sulfhydryl concentration increased 4.9%.

Discussion: Our hypothesis was to get decreased antioxidant defense at the second measurement. The reason of the unmet data is the meaningful changes of the patients dietary habits having known the cancer diagnosis. Based on this result we created a questionnaire to detect the supplementation intake of our patients. It seems to be that below 20% of patients didn’t change on the everyday lifestyle.

Conclusion: The measurement of influence of malignancy on redox homeostasis parameters holds several difficulties, one of these the uncontrolled supplementation. There is an urgent need to create precise measurement of the effect of any supplementation activity. It must be a personalized measurement for each patient.

Ethical Comittee Approval: Medical Research Council scientific and Research Committee Semmelweis University 2015/133
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Changes of Lipid Risk Factors in Women During and After Transition to Menopause.

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Key words: plasma lipids – cardiovascular risk - reproductive status

Introduction: Transition to menopausal status increases the risk for cardiovascular disease caused by atherosclerosis. One of proposed mechanisms are proatherogenic changes of plasma lipids. The aim of this study was to analyze longitudinally changes of lipid profile according to change of reproductive status.

Methods: Lipid spectrum including remnant lipoprotein cholesterol (RLP-C: total minus LDL minus HDL cholesterol in fasting status) was analyzed in 358 women around the age of menopause in 10-year longitudinal study. One hundred ninety-three women changed their menopausal status from premenopausal to menopausal and 165 were menopausal during the whole study. Impact of smoking status on the changes of lipids within both groups was also assessed.

Results: During follow-up, LDL cholesterol significantly increased in non-smoking women who changed their reproductive status to menopause. On the contrary, in smoking permanently menopausal women, LDL cholesterol decreased. Triglycerides and apolipoprotein B significantly increased only in non-smoking women who have transitioned to menopause; however, the absolute increase in triglycerides was small (median: +0.06 mmol/l, p=0.03), while more pronounced increase was detected for apolipoprotein B (median: +0.10 g/l, p=0.001). RLP-C significantly increased in non-smoking and smoking women who have transitioned to menopause, while it did not change in permanently menopausal women independently of smoking status. HDL cholesterol significantly decreased in all groups under study, while apolipoprotein A1 did not change significantly.

Discussion: We observed unfavorable changes of most of cardiovascular risk factors during 10-year follow-up in women who changed reproductive status to menopause, including increase of RLP-C. Surprising decrease of LDL cholesterol, not fully explainable by hypolipemic therapy, in smoking permanently menopausal women could be hypothetically caused by shift in lipid spectrum not detectable by standard methodology.

Conclusion: These findings support hypothesis that change of reproductive status could be one of the main accelerators of unfavorable lipid changes.

Acknowledgement: This project was supported through the Internal Grant Agency of the Ministry of Health, Czech Republic (NT 14008-3/2013) and under this number it was approved by local Ethical Committee in 2013.
Ultrastructural testicle of streptozotocin-induced diabetes of rat
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Key words: testis, hemomicrocircular channel, diabetes mellitus.

Introduction. Streptozotocin-induced diabetes in rats is frequently used to study the effects of diabetes in organs’ pathological changes like microangiopathy is considered an angiopathy of small vessels. The diabetes induced microvascular disease visible almost in all organs and tissues. The two most common forms are diabetic retinopathy and diabetic nephropathy and the pathophysiology is largely identical. Therefore, we investigate diabetic angiopathy in testicles.

Materials and methods. Streptozotocin dissolved in 0.1M citrate buffer, pH=4.5(7mg/100g) was administrated in 20 white male rats with single intraperitoneal injection aged 4.5 to 7.5 months (weight 130-150g). The diabetes progression was controlled according to glucose concentration in blood. The biological samples were taken every 2 weeks (after the control of glucose level in the blood, above 13.4 mmol/l), under thiopental anesthesia. The changes in the testicle vascular bed was performed with the injection of “Tempera” casein oil water black suspension. Morphometric analysis performed according the following quantitative criteria: (1) microcirculatory vessel diameter, (2) hemocapillaries packing density and (3) tissue trophic activity index. Evaluation performed in hispotahology samples. Statistical analysis was performed with «GraphPad InStar» packages - applied computer programs.

Results. After 2 weeks we see any remarkable changes. At 4 week of experiment: the electron optical density of capillary endotheliocytes decreased. The vessel’s wall swelling was intensified, separation of fibers and thickening of basement membrane was observed. In pericytes marginal location of nuclear chromatin and in mitochondria cristas broadening and fragmentation detected. In testicle arteriole endotheliocytes at the nucleus periphery with nuclear pores are noticed. Nevertheless, it is hard to define boundaries between condensed and uncondensed chromatin. The smooth muscle myocytes’ cytoplasm has average electron optical density; at the same time bundles of myofibrils partly disorganized. Arteriole adventitious coating is also swollen, thickened, with significant amount of amorphous liquid between bundles of collagen fibers. Venule lumens often got irregular, sometimes star-shaped form. Also mural thrombi found in the venules of small testicle.

Discussion. In rats with streptozotocin induced diabetes mellitus the morphological analysis of the testicle vascular bed allowed to demonstrate the change in vascularization state in the norm and diabetes mellitus. The two most common forms of diabetic angiopathy are retinopathy and nephropathy. The pathophysiologies are largely identical with diabetic angiopathy in rats’ testicles therefore it could provide a new diagnostic option.

Conclusion. Streptozotocin-induced diabetes in rats triggered angiopathy in the testicles. The results motivate morphologists and clinicians to investigate diabetic angiopathy in testicles as a new diagnostic opportunity.

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Ethical approval: University Animal Care and Use Committee Approval: № 8 of 18 November 2013.
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Breakaway Sessions

Common Mechanism Diseases
Saturday
10/08/2016
Obesity-induced insulin resistant rats: Interrelation between changes in leptin and cholesterol concentrations and key characteristics of glutamate- and GABA-ergic neurotransmission (project plan)

Dept of Lipid Research
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Using animal model of obesity-induced insulin resistant rats fed with prolonged high fat diet (58 % of fat for 6 months) we recently demonstrated that N-stearoylethanolamine restores pancreas lipid, composition (Onopchenko et al., Lipids, 2015). In the frame of this project we plan to determine leptin and cholesterol concentrations in obesity-induced insulin resistant young/elder rats and analyze whether or not these changes correlate with alterations in key characteristics of glutamate- and GABA-ergic neurotransmission.

Animal model: Obesity-induced insulin resistance was attained by feeding a prolonged high-fat diet (HFD) (58 % fat:23 % proteins:10 % carbohydrates for 6 months) as described (Svegliati-Baroni et al., 200). The amount of lipids in the diet was increased by addition of lard to the pellet diet, which contained a high level of palmitic (24 % of total FA) and stearic (28 % of total acids) acids. Cholesterol content of lard was not at a high level (0.57 mg/g of lard). The HFD–FA composition was at a ratio of 55 % saturated (SFA) to 45 % unsaturated FA (USFA). Control rats during the experiment were on normal pellet diet (4 % fat:23 % proteins:65 % carbohydrates) with SFA/USFA ratio 38 %/62 %, respectively. Throughout the experiments, the rats were gaining weight gradually. On the 24th week, the average weight of HFD rats was 410–430 g (due to visceral fat) in comparison with control rats of 330–350 g. Six months after HFD period, we conducted the oral glucose tolerance test.
Interaction of diphtheria toxin B-subunit with mammalian cells: potential for biomedical application

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Key words: CRM197, diphtheria toxin, endocytosis, HB-EGF, internalization.

Introduction The non-toxic derivatives of diphtheria toxin have a variety of clinical applications while their interaction with cells has not been characterized well enough. Recombinant subunit B of toxin has potential advantages over existing diphtheria toxin derivatives. The present study was aimed to analyze the pattern of binding and internalization of a recombinant diphtheria toxin subunit B in toxin-resistant and toxin-sensitive mammalian cells.

Methods The recombinant subunit B of diphtheria toxin was fused with enhanced green fluorescent protein (EGFP-SbB), expressed in E. coli, and purified by immobilized metal ion affinity chromatography. Binding and internalization of EGFP-SbB by resistant murine L929 fibroblasts and sensitive to toxin green monkey kidney epithelium Vero cells were characterized by flow cytometry and confocal microscopy.

Results L929 and Vero cells effectively bound and internalized recombinant subunit B of diphtheria toxin. According to the flow cytometry data, the binding constant (Kd) for EGFP-SbB interacting with L929 was approximately 0.372 μM, which is very similar to that observed for Vero (Kd = 0.269 μM). However, L929 cells bind 2.3 times less of EGFP-SbB when compared to Vero cells (Lbmax Vero/Lbmax L929). Dynamics of the endosome number, average size, and total area, calculated from the confocal cross sections of cells at different time points of incubation with EGFP-SbB, displayed a clear difference in subunit B internalization by L929 and Vero cells.

Discussion As both well-characterized standards of resistance and sensitivity, L929 and Vero cells internalize EGFP-SbB, the mechanism of the resistance is not determined by a ligand-receptor affinity and realized only after entrapment into endosomes. Lbmax reflects cellular proHB-EGF expression.

Conclusion Mammalian cells of different tissues and species absorb various quantities of the recombinant subunit B of diphtheria toxin; the examined cells have approximately the same Kd for subunit B. The processes of subunit B adsorption and intracellular endosomal transport clearly distinguish toxin-sensitive and toxin-resistant cells. The obtained data describe the kinetic parameters of subunit B interaction with mammalian cells in vitro and could be applied to the development of anti-tumour diphtheria toxoid-based treatment in humans. EGFP-SbB appears to be a suitable fluorescent probe for determining proHB-EGF levels and analyzing proHB-EGF-dependent endocytosis in cells.

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Ethical approval All the necessary precautions in terms of biosafety and bioethics have been met. The work did not involve chemicals, procedures or equipment that might have any harmful for the environment, impermissible or intolerable hazards inherent in their use. The study did not involve laboratory animals and human patients or volunteers
Nalbuphine: Some Aspects of the Research and Applications

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Key words: nalbuphine, opioid, addiction, morphine, experimental model

Aim To provide an analysis regarding available experimental models and approaches used in the research of opioid substances, in particular, in the researches of nalbuphine administration effects on organs morphology. To provide a comprehensive analysis of the risks and benefits associated with nalbuphine administration alongside with the current research of its mechanism of action.

Methods Searches in the electronic database (PubMed 1970 to 2015, Google Scholar 1970 to 2015) and hand searching in the local library were conducted. The searching criteria were: publication included cases of non-medical use of nalbuphine, general and clinical information about nalbuphine, information about the opioid mechanism of action, as well as experimental researches of morphological, physiological and biochemical changes under the effect of opioids (nalbuphine, first of all).

Results There 6 publication were found with recorded cases of the nalbuphine addiction from 1991 to 2014 (the number of proved cases of the nalbuphine addiction noted in publication are about 1740), there 47 publication were found with experimental data about morphological and biochemical changes under the effect of nalbuphine administration (includes 4 publication about liver changes, 5 – heart, 10 – eyeball, 2 –skin, 3 – pancreas, 11 – brain and nervous tissue, 3 – kidney, 7 – oral cavity, 6 – biochemical parameters).

Conclusions Nalbuphine hydrochloride causes well-expressed changes in the structural organization of the organs and systems. Drug addiction is one of the side-effects of nalbuphine administration. Despite a set of problems in clinical settings due to opioid nature of nalbuphine, it belongs to an indispensable group of analgesics for pain control.

Funding source None.

Ethical approval not required.
Selective inhibition of smooth muscle plasma membrane transport Ca\(^{2+}\),Mg\(^{2+}\)-ATPase by calix[4]arene C-90 and its activation by IPT-35 compound


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Aim: To investigate influence of selective plasma membrane Ca\(^{2+}\)-pump (Ca\(^{2+}\)-transporting Mg\(^{2+}\),ATP-dependent Ca\(^{2+}\),Mg\(^{2+}\),ATPase, PMCA) effectors – calix[4]arene C-90 (inhibitor) and IPT-35 (activator) - on Ca\(^{2+}\),Mg\(^{2+}\)-dependent ATP hydrolysis of plasma membrane, intracellular calcium homeostasis and myometrium smooth muscle strain contractions.

Methods: In this research methods of enzymology, mathematical modeling and physiological straining was used.

Results: It was shown that both effectors (100 µM) on the plasma membrane level selectively influence on PMCA enzymatic activity: calix[4]arene C-90 inhibit it on 75% and IPT activate on 40% relative to control meaning. Those compounds hardly influence on «basal» Mg\(^{2+}\)-independent Ca\(^{2+}\)-ATPase, Mg\(^{2+}\)-independent Ca\(^{2+}\)-ATPase and Na\(^{+}\),K\(^{+}\)-ATPase enzymatic activities. C-90 inhibition coefficient I\(_{0.5}\) meaning was about 20 µM and Hill coefficient n\(_{H}\) was 0.55. For IPT-35 activation constant A\(_{0.5}\) was 6.4 and Hill coefficient n\(_{H}\) was 0.7. Mathematical modeling indicated that implication of calix[4]arene C-90 to unexcited myocytes allow precisely change cytoplasm Ca\(^{2+}\) concentration, thus, influence on basal muscle tonus. The same method permits to determine that IPT-35 hardly had influence on Ca\(^{2+}\) concentration in unexcited myocytes. It was also shown that calix[4]arene C-90 in vitro can increase velocity of oxytocin-initiated contractions, whereas IPT-35 can suppress oxytocin-initiated velocity contractions in vitro.

Conclusion: Obtained results are promising for design of new pharmacological compounds – regulators of uterus contractions, namely, calix[4]arene C-90 can be perspective in obstetric in case of simultaneous use with oxytocin for enhancing uterus contractions, and IPT-35 may have antispasmodic effect on uterus contractility.

Ethics Statement: Experiments were carried out in accordance with the European Guidelines and International Laws and Policies. All procedures conformed to the guidelines of the Palladin Institute of Biochemistry. Before starting the experiments, the protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol №1 from 21/04-2014).

Funding: This work was supported by the Programs of National Academy of Sciences of Ukraine: “Molecular and cellular biotechnologies for medicine, industry, and agriculture” (Grant № 12-16, state registration № 0115U003639), “Fundamental problems of creation of new nanomaterials nanotechnologies” (Grant № 12-16, state registration № 0115U003638) “System biology” (Grant № 12-16, state registration № 0112U002624) and Cedars Sinai Medical Center’s International Research and Innovation Management Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars–Sinai Medical Center–RECOOP Research Centers (CRRC).
Consequences of perinatal hypoxia in developing brain: Changes in GABA transporter functioning in cortical, hippocampal and thalamic rat nerve terminals

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Key words: perinatal hypoxia, plasma membrane GABA transporters, nerve terminals, rat brain cortex, hippocampus, thalamus

Aim To analyze comparatively the initial velocity of transporter-mediated $[^3]$H]GABA uptake by cortical, hippocampal and thalamic nerve terminals isolated from rats of different age in control and after perinatal hypoxia.

Methods Wistar rat pups underwent hypoxia and seizures in an airtight chamber infused by atmosphere composed of 4% O$_2$ and 96% N$_2$ at the age of 10-12 postnatal days (pd 10-12). The experiments with rat brain cortical, hippocampal and thalamic nerve terminals (synaptosomes) were performed at pd 17-19, pd 24-26, pd 38-40 and pd 66-73 in the control (12 animals) and after hypoxia (12 animals).

Results The initial velocity of $[^3]$H]GABA uptake was higher in the young rats (pd 17-19) as compared to the older ones (pd 24-26, 38-40 and 66-73) in both control and hypoxia groups. It decreased abruptly by 50% in the thalamus and by 25% in the cortex for the period from pd17-19 to pd66-73. In the hippocampus, a decrease in the rate during the same time interval was 25%. Exposure to hypoxia had no effect on the intensity of $[^3]$H]GABA uptake in the cortex and thalamus, but caused a significant age-dependent attenuation (35%) of the uptake intensity in the hippocampus.

Conclusion Significant age-dependent hypoxia-independent decrease in GABA uptake with step-like dynamics of changes was shown in the thalamus and cortex. Gradual age-dependent hypoxia-dependent decrease in GABA uptake was revealed in the hippocampus, and so a larger vulnerability of this brain structure to hypoxia as compared to the cortex and thalamus was shown.

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Ethical Approval
All procedures were conducted according to the Declaration of Helsinki (“Scientific Requirements and Research Protocols” and “Research Ethics Committees”). Experimental protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol #1 from 19/09-2011).
**Antiplatelet and anti-proliferative action of disintegrin from the venom of Echis multisquamatis**

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**Keywords:** disintegrin, platelets, platelet aggregation inhibitor, snake venom, anti-proliferative action.

**Introduction.** Disintegrins are protein antagonists of cellular integrin receptors and may provide an effective approach for prevention of intravascular coagulation or be potent anti-proliferative agents. The aim of present work was the purification and characterization of the platelet aggregation inhibitor from *Echis multisquamatis* snake venom (PAIEM).

**Methods.** SDS-PAGE and MALDI-TOF were used for PAIEM identification. Platelet aggregation in the presence of PAIEM was studied on aggregometer Solar-AP2110; changes of shape and granularity of platelets in the presence of PAIEM were studied on flow cytometer COULTER EPICS XL, degranulation of platelets was estimated using spectrofluorimetry. Indirect ELISA was used for the determination of target of PAIEM on platelet surface. MTT-test was used to evaluation of the effect of PAIEM on proliferation of HeLa cells in cell culture.

**Results.** 14.9-kDa protein from *E. multisquamatis* venom was purified. IC_{50} of inhibitory effect of PAIEM on ADP-induced platelet aggregation was 7 nM. PAIEM did not affect thrombin- or ADP-induced platelet activation. In the same time it prevented binding of anti-IIb antibody to GPIIbIIIa-receptor of adhered platelets thus belonging to disintegrin family. PAIEM inhibited the viability of HeLa cells possibly by blocking their integrin interactions.

**Conclusions.** Highly specific direct antagonist of GPIIbIIIa platelet receptor was purified. It did not cause any changes in platelet shape and granularity during platelet activation and did not affect ADP-induced platelet degranulation. This disintegrin have been shown to be potent inhibitor of integrin-mediated cellular interactions including platelet aggregation or cancer cell proliferation.

**Funds:** This work was carried-out in the frame of the basic theme of the Palladin Institute of Biochemistry of NAS of Ukraine “Study of regulation mechanisms of blood coagulation and fibrinolysis interplay with vascular and platelet haemostasis”.

**Ethical approval** received from the ethics committee of the Palladin Institute of biochemistry of NAS of Ukraine (21.06.2016, N8).
Glu- and Lys-forms of plasminogen distinctly affect platelet aggregation, secretion and phosphatidylserine exposure

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Key words: plasminogen, platelet secretion, aggregation, phosphatidylserine exposure

Introduction. Plasminogen/plasmin system is known for its ability to support hemostatic balance of blood. However, plasminogen may be considered as an adhesive ligand and in this way affect the functioning of blood cells. The aim of this work was to investigate the influence of Glu- and Lys-form of plasminogen on the functioning of human platelets: aggregation, α-granule secretion and phosphatidylserine exposure.

Methods. Human platelets were obtained from human platelet-rich plasma by gel-filtration on Sepharose 2B. To estimate aggregation, platelets were stimulated with thrombin or collagen. Platelet secretion was studied using P-selectin antibodies conjugated with phycoerythrin. Phosphatidylserine exposure on the platelet surface was evaluated with FITC-conjugated annexin A5. Flow cytometry was used in both cases.

Results. Glu- and Lys-plasminogen have different impact on the platelet functioning. Exogenous Lys-plasminogen inhibits platelet aggregation, suppresses platelet α-granule secretion, but has no significant effect on phosphatidylserine exposure. Glu-plasminogen causes no effect on platelet aggregation but stimulates platelet secretion and increases phosphatidylserine exposure on the surface of thrombin- and collagen-activated human platelets.

Discussion. The obtained results showed the influence of plasminogen forms on the principal events of platelet functioning. Glu-plasminogen can be considered as a co-stimulator of agonist-induced platelet secretion and procoagulant surface formation. Meanwhile effects of Lys-plasminogen are probably directed at platelet-platelet interactions and not related to agonist-stimulated pro-apoptotic changes.

Conclusion. The observed different effects of Glu- and Lys-plasminogen on platelet aggregation, secretion and phosphatidylserine exposure can be explained by their structural peculiarities. The results should be taken into account at the treatment of cardiovascular patients, as both plasminogen forms appear to be natural modulators of the platelet function.

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Ethical approval. Research protocols were approved by the Ethical Committee of Palladin Institute of Biochemistry of NASU (from 3rd of November 2014, protocol N 10).
Ultrastructural changes of the lips' mucous membranes and the mouth corner and the links of their hermomiccirculatory flow in white rats at the late stages of experimental streptozotocin-induced diabetes

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Keywords: lips, mucous corner, rats, diabetes mellitus, capillaries.

Aim To provide analyses of the results of the experimental model and approaches used in the research of streptozotocine indused diabetes. To highlight the changes of capillary bed occurred at the result and describe effects on organs morphology.

Methods Male white adult rats (weight 100 - 130gr.) were taken. Animals were injected by srtepthozotocine single in a dose 7 mg/100gr. the biological samples (mouth angle and lip) were taken every two 2 weeks (after the control of glucose level in the blood), under thiopental anesthesia. Photographing of the material was carried out using a microscope UEMV - 100K (Ukraine ) at an accelerating voltage of 75 kV and the increase in the screen of 2000x Microscope - 12400h.

Results Thus, at the later stages of the pilot course of streptozotocin diabetes in the new record of epithelial mucosa of the mouth corner and lip we established a significant change in all cell layers. Installed reorganization of epithelial cells, destabilization of metabolic processes and effects on organs.

Conclusion This study yields new information about the processes and the pathogenesis of the dynamics of structural changes in the tissues studied and obtained at different times of the course of the experimentally simulated pathological process.

Funding source None

Ethical Committee Approval: was not provided!!!!!
The role of insulin resistance and altered structure of lipid rafts in neurodegenerative disorders

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1. Project summary
Alzheimer’s disease (AD) is characterized by insulin resistance in the brain that may contribute to neuronal cell death. Although the cause and mechanism of brain insulin resistance are only partly understood, its reversal can serve as a therapeutic target for treatment of neurodegeneration [1-3].
Previous studies demonstrated that AD is associated to uncoupling of insulin receptor (IR) activation and the downstream signaling in the brain [4-5]. As IR is situated in the lipid rafts, its function is significantly affected by this membrane microenvironment. In adipocytes altered composition of lipid rafts was reported to participate in the development of insulin resistance [6-7].
The role of chronic low grade inflammation in both peripheral and central insulin resistance as well as alteration of lipid raft structure was suggested [1-7].
As part of the Recoop “Diet induced obese prediabetic rat” project the effect of high-fat-high-sugar diet (HFHSD) on peripheral and central insulin resistance is aimed to be studied. These rats developed adipose tissue inflammation and systemic peripheral insulin resistance measured by HOMA-IR index but their central insulin signaling is not yet studied.
Responsiveness to insulin of peripheral and brain samples are to be studied on the basis of IR and insulin receptor substrate (IRS) phosphorylation and correlated to the alterations of lipid raft composition. Effect of antidiabetic treatments is also to be examined.
To further study the mechanism of brain insulin resistance and the involvement of lipid rafts in the process in vitro cell culture experiments would be carried out using neuronal cell line. In this latter we aim to find an in vitro model of central insulin resistance to examine its mechanism including alterations in lipid rafts as well as its role in the development of neurodegenerative disorders. Establishment of a model would help testing agents aimed to improve insulin sensitivity of neurons.

2. Background
In the eldering population of Western countries AD and other neurodegenerative disorders gain more and more importance. Development of effective therapeutic measures for these disorders requires better understanding their pathomechanism.
Low grade chronic inflammation and insulin resistance are increasingly reported as significant contributors of neuronal cell loss in neurodegenerative disorders and their reversal was suggested as a potential treatment [1-2, 4-5, 8-9].
Insulin resistance in the periphery is a well known feature of type 2 diabetes and prediabetic state. Although its mechanism is only partly understood, inflammation of adipose tissue plays a crucial role in its development. Proinflammatory cytokines, most importantly TNFalpha, induce various alterations that result in impaired responsiveness to insulin. Aberrant phosphorylation of IRS on serine and threonine residues was reported to be able to uncouple IR activation and downstream signaling pathway [10]. Accumulation of ganglioside GM3 in lipid raft or caveolae was also demonstrated in response to inflammatory stimulation that can render IRs to a less responsive membrane microenvironment and thus again decreases the activation of insulin signal transduction pathways [6-7].

The function of insulin in the brain is probably quite different from that in the periphery as glucose uptake into the neurons is insulin independent. Its neurotrophic growth factor role is more likely as its major signaling pathway, the PI3 kinase – Akt cascade is critical in the survival of brain cells. The brain insulin resistance is even less well understood than the peripheral one but the role of inflammatory cytokines and aberrant phosphorylation of IRS were suggested in the central nervous system as well [1-5].

3. Study plan

In the present research we aim to study the mechanism and connection of peripheral and central insulin resistance in HFHSD induced prediabetic state. The effect of antidiabetic treatments with metformin and liraglutide is also to be examined.

- Insulin induced IR and IRS phosphorylation in adipose tissue, striated muscle, liver and brain would be analyzed by western blot (2017 Q1-Q2)
- Cytokine levels in the brain would be studied by ELISA (2017 Q2)
- Ganglioside levels in the lipid rafts of the above tissues would be measured (2017 Q1-Q2)

To better characterize the mechanism of insulin resistance and test the effect of various agents in vitro cell culture experiments would be performed.

- Insulin induced IR and IRS phosphorylation in various models of neurodegeneration (neurotoxins, streptozotocin, inflammation) in neuronal cell lines would be analyzed by western blot (2017 Q3)
- Ganglioside levels in the lipid rafts isolated from the cells would be measured (2017 Q3-Q4)

4. Expected outcome

Better understanding the mechanism behind the insulin resistance of the brain could help finding interventions for its reversal that may have therapeutic value in Alzheimer’s disease.

References
5. **Costs of the study (USD)**

As the study is continuation of a previous animal experiment the majority of the costs involve the reagents to be used for western blot and ELISA analysis:

- Antibodies for IR, pIR, IRS, tyrpIRS, serpIRS, ganglioside: cca 250/vial, 2 vials each: 3,000 USD
- ELISA kit for cytokines: cca. 500/kit, 2 kits: 1,000 USD

Subtotal: 4,000 USD

6. **Justification of travel budget:**

Fruzsina Bagaméry, young scientist from Budapest would travel to Osijek, where the lipid raft isolation is an established method to learn and perform these experiments. Vedrana Ivic, young scientist from Osijek would travel to Budapest to participate in cell culturing experiments. Two 10-day periods would cost about 20x35 = 700 USD for accommodation and 2x50 = 100 USD for travel.

Subtotal: 800 USD

7. **Total requested support:** 4,800 USD

8. **Matching contributions:**

In kind contribution we can provide the laboratory facilities and consumables for analytical procedures and cell culturing. Consumables: Western blot reagents (gel, membrane, buffers, ECL reagent, films and photo reagents) and cell culture reagents (media, sera) and plastic ware (tissue culture plates and dishes). During mobility the young scientists will cover the cost of their meal.

Support is requested by the CRRC young scientists
Name and signature: Fruzsina Bagaméry

Collaborating young scientist:
Name and signature: Vedrana Ivic

Supervisors of the participating CRRCs agreed and the host CRRC declared the availability of matching fund.
Names and signatures: Tamás Tábi (Budapest)
Names and signatures: Marija Heffer (Osijek)

Department heads of the CRRCs
Names and signatures: Éva Szökő (Budapest)
Names and signatures: Marija Heffer (Osijek)
The role of oxidative stress in development of impaired vascular response in obese pre-diabetic elderly rats of both sexes treated with metformin or liraglutide

Applicant young scientist: Anita Cosic, M.Sc., PhD; Zita Tisza, PhD student
Applicant organization: Faculty of Medicine Osijek, University Josip Juraj Strossmayer Osijek
Supervisor: Prof. Ines Drenjancevic, M.D., PhD; Prof. Robert Gaspar, PhD

1. Project summary: This study aims to determine the effect of carbohydrate and fat rich diet (HFHSD) on vascular function of middle cerebral arteries (MCA) and oxidative stress in Sprague-Dawley (SD) rats of both sexes. Additionally, the effects of metformin (increases insulin sensitivity) and liraglutide (a glucagon-like peptide-1 receptor agonist who stimulates insulin secretion) will be examined in these conditions. SD rats will be divided into 4 test groups: control (standard diet for rats), HFHSD diet from 45 weeks (10 months) of age during 20 weeks (4 months)), HFHSD+liraglutide (carbohydrate and fat rich diet+treated with liraglutide 0.3 mg/kg/day s.c) and HSHFD+metformin (carbohydrate and fat rich diet+treated with metformin 50 mg/kg/day s.c.). Drugs will be given concomitantly to HSHFD from 51-65 weeks of age. At age of 65 weeks rats will be anesthetized prior to experiments with ketamine (75 mg/kg) and midazolam (2.5 mg/kg) and decapitated. Each animal will be weighed, blood glucose level and arterial blood pressure will be measured and serum levels of oxidative stress (TBARS) from arterial blood will be assessed. Flow-induced dilation (FID) of MCA will be assessed in vitro in the presence of acetylcholine (endothelium-dependent dilatation), SNP (endothelium independent dilation), tempol (superoxide scavenger) and inhibitors L-NAME (NOS inhibitor), INDOMETHACIN (inhibitor of COX's) and MSPPOH (inhibitor of CYP450 epoxidase reaction) separately or in combination. Protein expression of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, IL-6R, IL-1R and CCR-7 using Western blot from surface cerebral blood vessels. Since data on FID of MCA and the role of oxidative stress that may be altered by HFHSD are unexisting, tying the vascular functional studies with biochemical and molecular protein expression data are especially valuable for the understanding of these processes.

2. Background: Obesity is a serious health problem, as it increases the risk of morbidity from several pathologies, including hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, non-alcoholic fatty liver disease, osteoarthritis and etc [1].

Oxidative stress can be a consequence, but also a trigger of obesity related risks. Different dietary protocols, like high fat high carbohydrate (HFHC) diet, as well as high dietary saturated fatty acids (SFA) and trans-fatty acids stimulate intracellular pathways, leading to oxidative stress through multiple biochemical mechanisms, such as superoxide generation by NADPH oxidases (Nox), oxidative phosphorylation, glyceraldehyde autoxidation, protein kinase C (PKC) activation, polyol and hexosamine pathways [2-4]. Redox balance is generally evaluated by measuring markers of antioxidant defense and/or oxidative stress. Plasma concentrations of oxidation products (reactive oxygen species) and antioxidant enzyme activities are the most widely used biomarkers of the antioxidant state. In our preliminary data we have shown that there are sex-related differences in the level of oxidative stress and antioxidative enzymes activity in pre-diabetic obese elderly Sprague-Dawley (SD) rats. Metformin and liraglutide may modify antioxidative capacity more in female than male and also decreased activity of antioxidative enzymes in male rats [5].
3. Study plan:

**Step 1.** Diet protocol (02.01.2017. – 02.06.2017.) with or without liraglutide and metformin - standard diet and HFHSD (carbohydrate and fat rich diet) 20 weeks, both sexes, 8 per group.

**Step 2.** Data collection, functional and molecular studies (02.06.2017. – 02.11.2017.) for all experimental groups

**Step 3.** Data analysis and publication of results (02.11.2017. – 01.01.2018.)

4. Expected outcomes: To determine the differences in the effect of the HFHSD diet protocol on FID in MCA, determine the level of resulting oxidative stress, antioxidative enzymes, effects of liraglutide and metformin under these conditions, and sex differences in response to dietary protocol.

5. Costs of the study (USD):

- **Animal housing** – 12 rats (4 ♂ and 8 ♀) rats intended for breeding (1 rat = 18 $; 12 rats x 18 $ = 216 $); maintenance of animals (sawdust – 1 package = 30 $; 4 packaging/months x 120 $ = 1,680 $); standard rat food – 45 weeks (32 ♂ and 32 ♀ rats, ~0.05$ daily/rat-1008 $; (8 ♂ and 8 ♀ rats, ~0.05$ daily/rat (20 weeks)) 112 $; carbohydrate and fat rich food = 672 $ (24 ♂ and 24 ♀ rats, ~0.1$ daily/rat (20 weeks)) = 1,120 $; total: 3,688 $.
- **Anesthetics:** Midazolam- 110 $; Ketamine- 850 $; total: 960 $.
- **Consumables:** – consumables for sampling (sterile filter pipette tips, tubes) = 200 $; total: 200 $.
- **Metformin** - 86 $; Liraglutide - 114 $; total: 200 $.
- **Western blot:** chemicals for homogenization, Bradford solution for protein determination, primary and secondary antibody, PVDF membranes total: 2,000 $.
- **Functional studies – FID on MCA:** physiological salt solution (PSS) - NaCl 6$, KCl 11$, MgSO4 17$, CaCl2 9$, NaH2PO4 17$, NaHCO3 23$, EDTA 8$, glucose 11$ = 1025$; **inhibitors:** L-NAME 45$, INDO 50$, MSPPOH 50$ = 145 $; **other chemicals:** Ach 20$, SNP 10$, TEMPOL 370$ = 400 $; total: 647 $.
- **Oxidative stress – FRAP and TBARS:** Thiobarbituric acid (TBA) 25$, Trichloroacetic acid (TCA) 47$, 1,1,3,3-tetramethoxypropane (TMP) 55$, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) 69$, Iron(III) Chloride Hexahydrate (FeCl3 x 6H2O) 39$, sodium acetate trihydrate 11$ = 246 $.

6. Justification of the request for travel and accommodation support: All functional measurements and sampling will be performed in the Laboratory for Vascular Physiology and Laboratory for Molecular and Clinical Immunology at the Department of Physiology and Immunology, Faculty of Medicine J.J. Strossmayer University of Osijek, Croatia. After after sampling, Zita Tisza will take samples and do the WB in their laboratory (Szeged). We ensure accommodation for Zita Tisza on Faculty of Medicine.

Subtotal cost: **150 $** (meal + travel)

7. Total requested support (USD): **5,000 $**

8. Matching contribution (USD): **2,572 $**
Animal housing – 12 rats (4 ♂ and 8 ♀ rats intended for breeding (1 rat = 18 $; 12 rats x 18 $= 216 $); maintenance of animals (sawdust – 1 package = 30 $; 4 packaging/ months x 120 $= 1 680 $) - half of the price = 840 $; standard rat food – 45 weeks (32 ♂ and 32 ♀ rats, ≈0.05 $ daily/rat-1008 $, (8 ♂ and 8 ♀ rats, ≈0.05 $ daily/rat (20 weeks) 112 $ - half of the price = 560 $.

Anesthetics: Midazolam- 110 $ - half of the price = 55 $; Ketamine- 850 $ - half of the price = 425 $.

Consumables: – consumables for sampling (sterile filter pipette tips, tubes) = 200 $.

oxidative stress – FRAP and TBARS: Thiobarbituric acid (TBA) 25 $, Trichloroacetic acid (TCA) 47 $, 1,1,3,3-tetramethoxypropane (TMP) 55 $, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) 69 $, Iron(III) Chloride Hexahydrate (FeCl₃ x 6H₂O) 39 $, sodium acetate trihydrate 11 $ = 246 $. 

+ functional studies – FID on MCA: Ach 20 $, SNP 10 $ = 30 $ + storage of samples + Spacelabs Medical monitoring system for blood pressure measurements (Spacelabs Medical, Inc., Redmond, WA, USA) + Myograph Pressure System Model 110P MyoView Version 1.2.0 DMT for functional studies (Danish Myo Technology)

Support is requested by the CRRC young scientists: Anita Cosic, M.Sc., PhD

Zita Tisza, PhD student

Supervisors of the participating CRRCs agreed and the host CRRC declared the availability of matching fund: Prof.dr.sc. Ines Drenjancevic, MD, PhD

Prof. Robert Gaspar, PhD

Department heads of the CRRCs, name and signatures:

Prof.dr.sc. Ines Drenjancevic, MD, PhD

Prof. Robert Gaspar, PhD

References:

1. WHO. World Health Organization Fact Sheet for World Wide Prevalence of Obesity
The impact of obesity on pregnant rat uterine contractility: crosstalk of adipokines and Wnt proteins

**Applicant young scientist:** Judit Hajagos-Tóth, Ph.D.
**Applicant organization:** University of Szeged, Faculty of Pharmacy, Department of Pharmacodynamics and Biopharmacy
**Supervisor:** Róbert Gáspár

**Collaborating CRRC organization:** Department of Cellular Proliferation & Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine.
**Collaborating young scientist:** Finiuk Nataliya, Ph.D.
**Supervisor of the young scientists collaborating CRRC organization:** Olexandr Korchynskyi

1. **Project summary:**
The increasing prevalence of maternal obesity is a major public health concern. It is associated with increased incidence of caesarean section, pregnancy induced hypertension, postpartum hemorrhage. Obesity reduces fertility, and in pregnancy it is associated with a heightened risk of gestational diabetes mellitus and failure in uterine contractions. The underlying mechanisms by which obesity impairs ovarian/embryonic/endometrial function remain incompletely understood.

It is known that some adipokines (leptin and visfatin) that have increased levels in obesity reduce the contractility of human or rat myometria. It was also established that in rats, gestational obesity increases the intensity of spontaneous contractions of the last-day pregnant uterus, but reduces its sensitivity to oxytocin. Elevated level of adipokines also can lead to dysregulation in Wnt/β-catenin, bone morphogenetic proteins (BMP) activation and IGF1 signals resulting in pregnancy pathology and offspring diseases. These findings clearly suggest that gestational obesity via increased level of adipokines induces both preterm and postponed labor, and can lead to the development of diseases later in the children. The main questions are the myometrial effects of different neuropeptides (leptin, adiponectin and kisspeptin) and their inhibitors and to determine their receptors in the pregnant rat myometrium throughout gestation. Our further aims are to investigate the level of key Wnt proteins (Wnt1, Wnt3a, Wnt4) in a combination with BMP-Smad activation and IGF1 levels. Our results may provide a better understanding of the mechanism of abnormal myometrial contractility during maternal obesity, as the underlying causes and molecular pathways have not been fully elucidated. Therefore there is a need for investigations of endogenous factors that might control uterine activity, with the perspective for the successful family planning.

2. **Background:**
Obesity increases the mass of the fatty tissue. It is known that the adipocytes are producing several cytokines: adipokines (e.g. leptin, adiponectin), proinflammatory modulators (e.g. IL-6, TNFα) and other peptide products (e.g. kisspeptin). The majority of these products have impact on gonadotropins and fertility (Hersh et al. 2015). Although the peripheral actions of several adipocyte products have been investigated, the results of these studies reveal significant inconsistencies and methodological limitations regarding the direct effects of these adipokines on peripheral reproductive tissues including uterine tissue (Kawwass et al., 2015). Well-designed studies are required to clarify how the obesity may induce both preterm and postponed labor and one of the keys should be hidden in the relationship between adipocyte products (adipokines, cytokines and other products) and uterine contractility. Wnt proteins are key morphogens that control organ and general body pattern through embryonic and postnatal development and tissue homeostasis. Several human diseases are
linked to dysfunction of these signal mediators. Targeted inactivation of the key Wnt proteins and of cooperating BMP proteins leads to early embryonic death due to malformations in mesenchymal tissue and blood vessels (Korchynskyi et al., 2003).

3. Study plan:

Step 1: Determination of myometrial expression of leptin, adiponectin and kisspeptin receptors in normal and obese pregnant rats on pregnancy days 15, 18, 20 and 22.

Step 2: Comparison of the plasma levels of these adipocyte products with their uterine levels.

Step 3: Localizations (endometrium, myometrium) of the receptors.

Step 4: Investigation of the uterine contractility of normal and obese pregnant rats in vitro on pregnancy days 15, 18, 20 and 22.

Step 5: Investigation of the effects of agonists and antagonists of the selected receptors on the contractility of normal and obese pregnant rat uterus in vitro.

Step 6: Determination of the Wnt1\Wnt3a\Wnt4 proteins and IGF1 profiling will be done using plasma samples from the mother and newborns to reveal differences. Transcriptome analysis of placenta samples after tissue homogenization in a combination with the control of β-catenin accumulation (marker of Wnt pathway activation) and BMP R-Smad1/5/8 phosphorylation will also be performed.

4. Expected outcomes:

4.1. Better understanding the influence of obesity on pregnant uterine activity. Our animal model helps to clarify how the obesity alters the uterine contractility in a controlled way. The results may reveal the impact of gestational obesity on contractions when overweight is the sole risk factor during pregnancy.

4.2. Clarification of the roles of adipocytes products in pregnant uterine activity. The knowledge about the fatty tissue-secreted hormones is increasing, but there is still little known about their impact on pregnant uterus. Our project promises to find answer how the leptin, adiponectin and kisspeptin system alter the function of pregnant uterus and how their roles and actions are altered in obese pregnancy. The detection of plasma levels of these peptides may give a chance to find biomarkers to predict increase or decrease in pregnant uterine contractility.

4.3. Discovery of new targets to prevent premature birth or prolonged pregnancy. The adipocytes-produced peptides and their receptors may serve as new targets for drug development to reduce the risk of premature birth or prolongation of gestational period. Both disorders are relevant clinical problems without appropriate pharmacotherapy. Obesity can induce both and we assume that in the pathophysiology of these disorders the adipocytes-secreted peptides and their receptors may have a crucial role. Our project may reveal important correlations between these systems and the uterine activity.

4.4. Maternal obesity or even father’s obesity via elevation of adipokines lead to dysregulation in Wnt/β-catenin and IGF1 signals which can lead to the development of maternal and offspring bone and joint diseases, such as rheumatoid arthritis. Determination of the differences in the maternal and newborn Wnt proteins and IGF1 level may give a chance to find biomarkers to predict these chronic disorders or may reveal new pharmacological targets.

5. Costs of the study (USD):

Animal housing: in kind contribution
Laboratory analyses: reagent kits Wnt proteins: 1500
Consumables: antibodies for Western blot, primers for RT-PCR, and adipokines agonist and antagonists cost: 2640
Other: 0
Subtotal cost: 4140

6., Justification of the request for travel and accommodation support:
The collection of placenta from the mothers and plasma samples from the mothers and new borns for the Wnts and IGF1 profiling will be carried out in Szeged, therefore we request support for the travelling and accommodation of the Ukrainian participant, Nataliya Finiuk, Dept. of Cellular Proliferation & Apoptosis, Inst. of Cell Biology, Natl. Acad. Sci. of Ukraine.

Requested support: itemized like
Travel expenses (USD): from Lviv to Szeged and back: 100 (train tickets)
Meals (USD): 400
Accommodation (USD): price/nights x total nights and indicate the location: Szeged, 60 USD/ nights, 6 nights: 360 USD
Subtotal cost: 860 USD

7., Total requested support (USD): 5,000

8., Matching contribution (USD): $ 2,500 please itemize
The matching fund is the PhD research program fund of Department of Pharmacodynamics and Biopharmacy
In kind contribution: laboratory animals, animal housing, laboratory reagents and tubes

Support is requested by the CRRC young scientists
Name and signature
JUDIT HAJAGOS-TÓTH

Supervisors of the participating CRRCs agreed and the host CRRC declared the availability of matching fund.
Names and signatures
RÓBERT GÁSPÁR

Department heads of the CRRCs, name and signatures
RÓBERT GÁSPÁR

Partner CRRC young scientist
Name and signature
NATALIYA FINIUK

The matching fund is, State Fund of Fundamental Research of Ukraine. Project title: Generation of Novel Bioactive Osteoplastic Materials That Contain Bone Morphogenetic Proteins for Osteogenesis Induction PI: O.Korchynskyi
In kind contribution: laboratory reagents for detection of BMP pathway activation.
Supervisors of the participating CRRCs agreed and the host CRRC declared the availability of matching fund.
Names and signatures
OLEXANDR KORCHYNSKYI

Department heads of the CRRCs, name and signatures
ROSTYSŁAV STOIKA
Correlation between placental vascularization indices and sFlt-1/PlGF ratio in preeclampsia screening.
Applicant young scientist: Ábel Tamás Altorjay MD
Applicant organization: Department of Obstetrics and Gynecology, University of Szeged, Hungary
Supervisor: Andrea Surányi MD, PhD
Collaborating CRRC organization: Faculty of Medicine, University J. J. Strossmayer Osijek, Croatia
Collaborating young scientist: Martina Vulin, MD
Supervisor of the young scientists collaborating CRRC organization: Andrijana Müller MD, PhD

1. Project summary: Incidence of preeclampsia (PE) is the most serious hypertensive disorder during pregnancy. It occurs in 3-5% of pregnancies and is defined by new onset maternal hypertension and the coexistence of one or more of the following new-onset conditions: proteinuria, renal insufficiency, liver involvement, neurological, haematological complications or foetal growth restriction. PE may develop from 20 weeks gestation until 48 hours after delivery. It is most commonly diagnosed after 32 weeks of gestation. Early onset PE (20-32 weeks) is associated with particularly serious threats for the mother and fetus. PE has the greatest effect on maternal and infant outcome. It is a leading cause of preterm birth and consequent neonatal morbidity and mortality. Hypertension and the coexisting conditions are the diagnostic criteria for PE but they are only symptoms of the pathophysiologic changes that occur in the disorder. Therefore screening of PE is a highlighted task during pregnancy care.

The aim of the present study is to identify for the first time the correlation between placental vascularization indices measured by 3-dimesional power Doppler (3-DPD) and soluble fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio in PE. Our goal is to understand the background of PE through molecules that are essential in angiogenesis and vasculogenesis as sFlt-1 and PlGF, and to identify their importance in placental development, through the measurement of placental 3-DPD vascularization indices (such as: vascularization index VI, flow index FI, and vascularization flow index VFI).

Our purpose is to measure sFlt-1/PlGF ratio through blood samples in second and third trimester, and to monitor placental vascularization indices every four weeks between 20th and 36th weeks of gestation in pregnancies with high risk for PE.

From our point of view this study will add essential information to our recent knowledge for the better understanding of the pathophysiological background and for the better clinical management of PE cases. It is important to find the link between laboratory data and clinical observational study data to improve detection rates, treatment success and nevertheless patient safety especially in case of pregnancy, as we are dealing with not one but two patients (mother and neonate) at the same time!

2. Background: The aim of pregnancy examinations is the early detection of specific risks. Risk screening using multiple markers has achieved wide acceptance in routine antenatal care. Non-invasive screening methods in the first and second trimester for detection of placental abnormalities have been developed over the last 15 years. Due to benefits of the earlier decision process there is a clear trend towards first trimester screening. PE is a serious complication in pregnancy which affects both the mother and the unborn child. In the majority of cases PE develops in healthy women bearing their first child. In addition several medical conditions are associated with an increased PE risk such as chronic hypertension, diabetes and renal disease. During fetal development, the human placenta undergoes high levels of both angiogenesis and vasculogenesis. The initiation, maturation, and maintenance of the placental vasculature are of critical importance. Recent studies showed that the first step in the building of the vascular network is mediated by the vascular endothelial growth factor (VEGF) family, which includes...
PIGF and the VEGF family receptor sFlt-1. PIGF is expressed in the placenta and is a proangiogenic while sFlt-1 binds PIGF and inhibits its activity.

The cause of PE is not fully understood, but there is growing evidence that angiogenic growth factors such as PIGF and sFlt-1 play a major role in the development of PE. PIGF is responsible for normal placental function and thereby for the maintenance of healthy pregnancy, whereas sFlt-1 is associated with termination of pregnancy in the last weeks of gestation. In pregnancy hypertension patient’s sFlt-1 level is increased prior to clinical symptoms and also correlates with the severity of the disease, while PIGF levels are significantly decreased. Recent clinical studies suggest that sFlt-1 and PIGF, especially when sFlt-1/PIGF ratio is calculated, may serve to diagnose and predict PE and its related complications.

Proper uterine and placental vascularization is important for the adequate development of pregnancies. Pathological foetomaternal circulation can lead to elevated resistance in uterine circulation, which can cause placental insufficiency and thus - due to pathological development of the placenta - result in premature birth, intrauterine hypoxia, or even intrauterine death.

We would use Mercé-type placental sonobiopsy, a reproducible, validated method for obtaining representative sample of the placental tree, which - contrary to other methods - is applicable throughout the whole pregnancy. In a placental virtual sample we will analyse the 3-DPD indices by VOCAL (virtual organ computer aided analyses) program.

3. Study plan:
   **Step 1.** Patient recruitment: From January 2, 2017 to August 31, 2017 we would select patient at high risk for PE in Department of Obstetrics and Gynecology in Szeged (HU) and Osijek (HR). Increased likelihood thus including criteria of PE would be: previous PE; pre-existing medical conditions such as chronic hypertension, gestational hypertension, underlying renal disease, or pre-gestational diabetes mellitus; underlying antiphospholipid antibody syndrome. Inclusion and exclusion criteria will be made based on consensus and the study group in Osijek will adopt and follow the protocol of the study group in Szeged.
   **Step 2.** Blood test: Between January 2, 2017 and August 31, 2017 we would take blood samples from each participant at second and third trimester for sFlt-1/PIGF ratio measurements performed at Department of Laboratory Medicine, University of Szeged.
   **Step 3.** Ultrasound, blood pressure measurements and lab tests: From January 2, 2017 to November 30, 2017 we would perform ultrasound examinations of the placenta every four weeks between 20th and 36th weeks of gestation by 3-dimensional method. We would also perform measurements of blood pressure, proteinuria, lab tests for renal insufficiency, liver involvement, neurological or haematological complications in each occasion.
   **Step 4.** Data analysis: In December 2017 we would start to analyze the data extracted from ultrasound (3DPD data will analyzed by VOCAL) and laboratory measurements and from the data collected from pregnancy outcomes of PE cases.
   **Step 5.** Presentation of the results/publication: We would like to present our study results in the XXI World Congress of International Society of the Study of Hypertension (ISSHP) in Amsterdam 2018, and immediately after we would like to start the publication process.

4. Expected outcomes: Our opinion is that the present study will provide essential information to our recent knowledge on the pathophysiological background of PE through the exploration on the correlation between sFlt-1/PIGF ratio and 3-DPD indices. These results may be useful in deeper understanding the background mechanisms, and the possibility of earlier detection of PE.

5. Costs of the study:
   Markers/reagents:
PLGF Elecsys cobase e 100 100 pieces 2.273,- USD
PLGF CS Elecsys 4x1 ml 207,- USD
sFLT1 Elecsys cobase e 100 100 pieces 2.273,- USD
sFLT1 CS Elecsys 4x1 ml 207,- USD
PreciControl Multimarker Elecsys 2x3x2 ml 160,- USD
Subtotal cost: 4.150,- USD

6. Justification of the request for travel and accommodation support:
In first phase of the project (between Januar and March, 2017) during one week joint research training of a clinician from Osijek will learn our research methods: adopt the patient recruitment protocol, learning about how to perform placental 3-DPD measurements and data records. The young scinetist, who is supported by BH RECOOP YS grant from March to May: confirming the placental 3-DPD measurements and data records, discussing the experiences gained from the study, the results and future cooperation possibilities.
Travel expenses: from Osijek to Szeged and back /220km/ 250,- USD
from Szeged to Osijek and back 250,- USD
Accomodation expenses: 35,- USD/nights * 10 350,- USD
Subtotal cost: 850,- USD

7. Total requested support: 5.000,- USD

8. Matching contribution:
We already own 100 pieces of PLGF and sFLT1 cobase kit. The value of it is 5.120,- USD. We would also add 970,- USD to cover the expenses of the reagents will be bought from our financial source.

Support is requested young scientist: Ábel Tamás Altorjay MD

Supervisor of the participating organization and the host declared the availability of matching fund Andrea Surányi MD, PhD

Gábor Németh MD, PhD
Chairman, Department of Obstetrics and Gynecology, University of Szeged, Faculty of Medicine, Hungary

Collaborating CRRC organization

Andrijana Müller MD, PhD, Department of Obstetrics and Gynecology, University Hospital Center Osijek,
RECOOP Visegrad Scholarship Program

Visegrad Scholarship [http://visegradfund.org/scholarships/](http://visegradfund.org/scholarships/)

The top ten young scientists selected during the Bridges in Life Sciences Annual Conferences have the opportunity to apply for International Visegrad Fund (IVF) Scholarship and receive the RECOOP Young Scientists Matching Fund. The Visegrad Scholarship is the Visegrad Four European Macro-Region’s Fulbright Program. Therefore it could be important to link the Visegrad Scholarship and the Fulbright Foreign Student Program.

RECOOP HST Association in 2014 won two Visegrad Scholarships:

**Post-Master’s Scholarship:**

Ivana Koborová  
Institute of Molecular Biomedicine  
Medical Faculty, Comenius University, Bratislava, Slovakia  

Research project at the Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary from September 2014 to January 2015:

“How relationship of SSAO/VAP1 and insulin resistance in adolescents”

**In-Come Scholarship:**

Alexander Karmash  
Intern at the Department of Regulation of Cell Proliferation and Apoptosis  
Institute of Cell Biology, NASU, Lviv, Ukraine  
(Department of Biochemistry, Ivan Franko Lviv National University, Ukraine)

Research project at the Horváth Laboratory of Bioseparation Sciences at the Research Centre for Molecular Medicine, University of Debrecen, Hungary, September 2014 – January 2015:

“Role of disease-related changes in immunoglobulin IgG glycosylation”

**Visegrad Scholarship Program (VSP)**

The International Visegrad Fund offers Master’s and Post-Master’s scholarships awarded to selected scholars for periods of 1 or 2 semesters (with the exception of Master’s scholarships within the Visegrad Scholarships schemes where 1– to 4-semester scholarships can be awarded).

The following scholarship schemes are available:

- Intra-Visegrad Scholarships
- In-Come Scholarships
- Out-Going Scholarships
- Scholarship Program for Belarusian Students
Scholarship Program for Ukrainian Students
Visegrad Scholarships at OSA Archivum (separate program)
If selected each scholar receives the scholarship funding at the beginning of each five-month period (semester) upon a written confirmation from the host university/institution.
Deadline for all scholarship applications is 31 January. Results are announced by mid-May.

CSMC – RECOOP Research Centers (CRRC) the Center of Excellences of the RECOOP HST Association. They host young scientists, Ph.D. students with CSMC – RECOOP (IVF – CSMC - RECOOP) Scholarship. The RECOOP HST Association Scientific Advisory Board selects the young scientists who could compete for IVF – CSMC - RECOOP Scholarship.

The selected young scientists (preferably Ph.D. students) will spend maximum four semesters at the host organization and receive: €2,300 / semester and the corresponding host universities/institutes receive €1,500/semester/scholar. The host CRRC will get $1,500 for laboratory expense and consumables from CSMC – RECOOP HST Association. Applicants whose current (i.e. at the time of applying) university or employer is further than 1,500 km from the selected host university/institute are eligible for a one-time travel grant.

RECOOP HST Association Members from the Visegrad Group Countries:
IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic
University of Debrecen, Hungary
University of Pecs, Hungary
University of Szeged, Hungary
Slovak Medical University, Bratislava, Slovakia

RECOOP HST Association Member Organizations allegeable for the In-Coming scheme
Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine
Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

RECOOP HST Association’s Cedars – RECOOP Research Center (CRRC) could participate
Semmelweis University, Budapest, Hungary
Comenius University in Bratislava, Slovakia
Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland
University Hospital in Hradec Kralove, Czech Republic
CROATIA

School of Medicine, Josip Juraj Strossmayer University of Osijek

Professor Ines Drenjancevic, MD, PhD,
Department of Physiology and Immunology
Vice Dean for Science, Faculty of Medicine Osijek (since 2005)
University J. J. Strossmayer Osijek
Honorary university professor, University of Pecs, Hungary (since 2012),
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