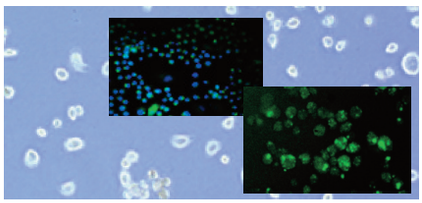


DEPARTMENT OF PATHOPHYSIOLOGY AND PHARMACOLOGY

***Adriana Georgescu**, PhD*
HEAD OF DEPARTMENT

STAFF

***Gabriela Tanko,** PhD*
***Nicoleta Alexandru - Moise,** PhD*
***Alina Constantin,** PhD*
***Miruna Nemecz,** PhD*
***Alexandru Filippi,** PhD*
***Marilena Isachi,** Technical assistant*
***Ioana Karla Comarita,** Master student*
***Anastasia Procopciuc,** Master student*
***Alexandra Vîlcu,** Master student*

FORMER RESEARCH STAFF:

Doina Popov, *PhD*
/Academician, Head of the Department 1979-2016/
Mirela Enache, Ana Maria Grigore, Mirela Hasu,
Alexandra Vulpanovici, Mădălina Dumitrescu, Marcela Toader.

CORE LABORATORY UNITS:
Myography
responsible
Adriana Georgescu

Laser Microdissection
responsible
Gabriela Tanko

DEPARTMENT OF PATHOPHYSIOLOGY AND PHARMACOLOGY

Adriana Georgescu, *PhD, Habil*
Head of Department
E-mail: *adriana.georgescu@icbp.ro*

Major positions and appointments

- Principal Investigator Grade I
- Member of the Scientific Council of ICBP 'Nicolae Simionescu'
- Ph.D. Coordinator
- Supervision of Graduate Students and Postdoctoral Fellows
- Expert Evaluator of the Biology, Biochemistry and Pharmacy Commission of the National Council for the Certify of Titles, Diplomas and University Certifications (CNATDCU) (2012)
- Expert Evaluator of the National and International Grants
- Peer Reviewer for International Scientific Journals

MAJOR RESEARCH INTERESTS

- **Microparticles as biomarkers, biological vectors for microRNAs and potential biomedical tools modulating atherosclerosis: role of the extracellular vesicles released from adipose tissue-derived stem cells and bone marrow mesenchymal stem cells in the macrophage polarization, and reversion of endothelial dysfunction and cardiac hypertrophy**
- **Endothelial progenitor cell dysfunction and platelet-endothelial progenitor cell interplay in atherosclerotic disease**
- **Atherosclerosis-associated diabetes mellitus and aortic valve disease: relationships between plasma biochemical parameters, circulating endothelial progenitor cells and early structural/functional alterations; establishing potential therapy**
- **Diabetes mellitus and obesity - associated cardiovascular alterations: microRNA expression profile in disease development, macrophage involvement in pancreatic beta cell response to metabolic stress**

PUBLICATIONS

Over 110 original articles (>2300 citations) were published in Web of Sciences Core Collection journals, 3 books and 14 book chapters between **1979-2019** by researchers of the Pathophysiology and Pharmacology Department.

SELECTED NEW FINDINGS OF THE DEPARTMENT

• In experimental hyperlipemia-hyperglycemia administration of L-arginine improves the microangiopathic changes of coronaries and enhances vasodilation of resistance arteries

• Enoxaparin restores the vascular reactivity of resistance arteries in ageing and diabetes

• Nebivolol has a reversible vasodilator effect on renal arteries

• Obesity alone or obesity associated with Type 2 diabetes alters human periumbilical adipose tissue arterioles in terms of structure, function and biochemistry, including diminished eNOS expression and reduced levels of IRS-1, IRS-2, PI3K and Akt in the insulin signaling pathway.

• Sera of obese type 2 diabetic patients undergoing metabolic surgery instead of conventional treatment exert beneficial effects on beta cell survival and function.

• Treatment with free fatty acids designed to limit oxidative stress, endoplasmic reticulum stress, inflammation and apoptosis may point toward novel strategies for improving beta cell function under saturated conditions.

• CO_2 laser increases the regenerative capacity of human adipose-derived stem cells by a mechanism involving the redox state and enhanced secretion of pro-angiogenic molecules.

• In a hypertensive-hypercholesterolemic hamster model, the endothelial progenitor cell-based therapy suppresses the development of atherosclerosis and reduces hepatic lipid and macrophage accumulation with the consequent alleviation of dyslipidemia and hypertension.

• Irbesartan administration therapeutically influences platelets activation, circulating endothelial progenitor cell and microparticle mobilization by involvement of pro-inflammatory cytokines in an atherosclerotic animal model and in patients with hypertension and dyslipidemia.

• Microparticles (microvesicles) and platelets of healthy origins improve atherosclerotic endothelial progenitor cell dysfunction via microRNA transfer in a vitro model.

• Allogenic microvesicles administration of healthy origins to an atherosclerotic animal model (hypertensive-hypercholesterolemic hamster), can counteract diet induced detrimental effects on plasma, structural and functional parameters by biologically active miR-10a, miR-21, miR-126, miR-146a transfer to circulating endothelial progenitor cell mediating their vascular repair function in atherosclerosis processes.

• Early aortic valve dysfunction was detected by echocardiography after one week of diabetes in a murine model of atherosclerosis.

• The ratio between circulating microvesicles and endothelial progenitor cells as potential biomarker in hypertensive-hypercholesterolemic patients.

• Circulating microvesicles containing pro- or anti-angiogenic microRNAs play a key role in the development of vascular complications in patients with type 2 diabetes.

PREVIOUS RESEARCH PROJECTS/ RELEVANT PUBLICATIONS

• **Biochemical mapping of endothelial cell surface: evidence for differentiated microdomains** (Ghinea N. and Simionescu N., J. Cell Biol 1985; Ghinea N. et al., J Submicrosc Cytol Pathol 1987; Leabu M. et al., J Submicrosc Cytol Pathol 1987)

• **Detection of Albumin Binding Proteins** (Ghinea N. et al., J Cell Biol 1988; Popov D., J Mol Cell Cardiol 1992)

• **Interaction of AGE-albumin with normal and diabetic capillary endothelium** (Schmidt A.M. et al., Proc Natl Acad Sci USA, 1994; Popov D. and Simionescu M. Arch Physiol Biochem 2006; Simionescu M. et al., Cell Tiss Res 2009)

• **Pathomorphological changes of micro- and macrovasculature in diabetes** (Popov

D. et al., Acta Diabetol 1996, 1997; Simionescu M. et al., Am J Pathol 1996; Popov D. and Simionescu M., Eur Respir J 1997; Mompeo B. et al., J Submicrosc Cytol Pathol 1998; Costache G. et al., J Submicrosc Cytol Pathol 2000; Popov D. et al., Cell Tiss Res 2002; Popov D. et al., Diabetologia 2003)

- **Vascular reactivity of resistance arteries in hyperlipemia associated with hyperglycaemia** (Georgescu A. and Popov D., J Am Aging Assoc 2000; Georgescu A. et al., Fundam Clin Pharmacol 2001, 2003; Georgescu A. et al., Vasc Pharmacol 2003, 2006)

- **Mechanisms involved in nebivolol effects on renal artery in diabetes associated with hypertension** (Georgescu A. et al., Eur J Pharmacol 2005, 2008; Georgescu A. et al., Pharmacology 2007, 2008)

- **Polymorphisms of the leptin and leptin receptor gene** (Constantin A. et al., Biochem Biophys Res Commun 2010)

- **Effect of high glucose concentration/ diabetes mellitus on human blood platelets** (Alexandru N. et al., Platelets 2007; Alexandru N. et al., J Cell Mol Med 2008; Alexandru N. et al., Clin Chem Lab Med 2008, Alexandru et al., Trends Cardiovasc Med 2010)

- **Contribution of circulating microparticles and platelets to human peripheral venular dysfunction: focus on chronic venous insufficiency** (Georgescu A. et al., J Thromb Haemost 2009; Alexandru N. et al., Clin Lab 2011)

- **Dysfunction of human subcutaneous fat arterioles in obesity alone or obesity associated with Type 2 diabetes** (Georgescu A. et al., Clinical Science 2011)

- **Circulating microparticles, endothelial progenitor cells and platelet activation in atherosclerosis; effects of irbersartan** (Alexandru N. et al., J Thromb Haemost 2011; Georgescu A. et al., J Thromb Haemost 2012; Alexandru N. et al., Thromb Res 2012; Alexandru N. et al., PloS One 2013; Georgescu A. et al., Eur J Pharmacol 2013; Badila E. et al., Farmacia 2014, Andrei E. et al., Exp Clin Cardiol 2014)

- **Studies designed to find new and better ways to treat patients with diabetes: PTP1B protein expression in human aortic smooth muscle cells exposed to high glucose concentration; enoxaparin effects on adrenergic contraction of resistance arterioles; activation profile of dorsal root ganglia Iba-1 (+) macrophages** (Popov D. et al., Biochem Biophys Res Commun 2009; Georgescu A. et al., Blood Coagul Fibrin 2011; Thi Ton B-H et al., Acta Histochem 2013; Badila E. et al., Eur J Pharmacol 2015)

- **CO_2 laser increases the regenerative capacity of human adipose-derived stem cells by a mechanism involving the redox state and enhanced secretion of pro-angiogenic molecules** (Constantin A. et al., 2017)

- **Endothelial progenitor cells - based therapies on vascular dysfunction in diabetes and atherosclerosis** (Georgescu A. et al., Eur J Pharmacol 2011; Georgescu A. World J Diab 2011; Alexandru N. et al., Biol Cell 2015; Georgescu A. et al., Biol Cell 2016)

- **Circulating microparticles and microRNAs as biomarkers and diagnostic tools in hypertension, atherosclerosis and diabetes** (Sadri C. et al., Diabetes 2010; Alexandru N. and Georgescu A. World J Hematol 2013; Orbe J et al., Thromb Res 2015; Alexandru N. et al., Biochem Biophys Res Commun 2016; Nemecz et al., Curr Hypertens Rep 2016; Alexandru N. et al., Curr Stem Cell Res Ther 2017; Stępień EŁ. et al., Theranostic 2018; Georgescu A. et al., Acta Physiol 2018)

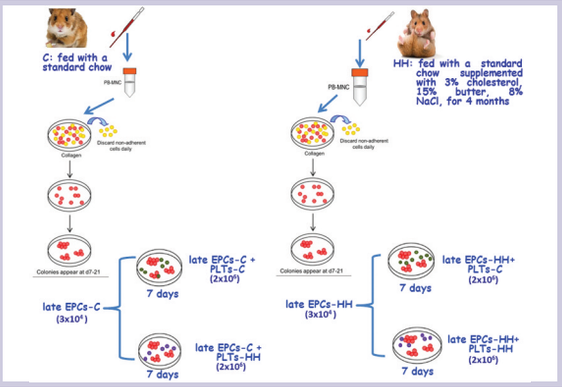
- **Microparticles and platelets of healthy origins improve endothelial progenitor cell dysfunction via microRNA transfer in an atherosclerotic hamster model** (Alexandru N. et al., Acta Physiol 2017)

CURRENT PROJECTS

1. MICROPARTICLES (OR MICROVESICLES) AS INTRACELLULAR DELIVERY STRATEGIES FOR MICRORNAS AND POTENTIAL THERAPIES FOR ATHEROSCLEROTIC VASCULAR DISEASE

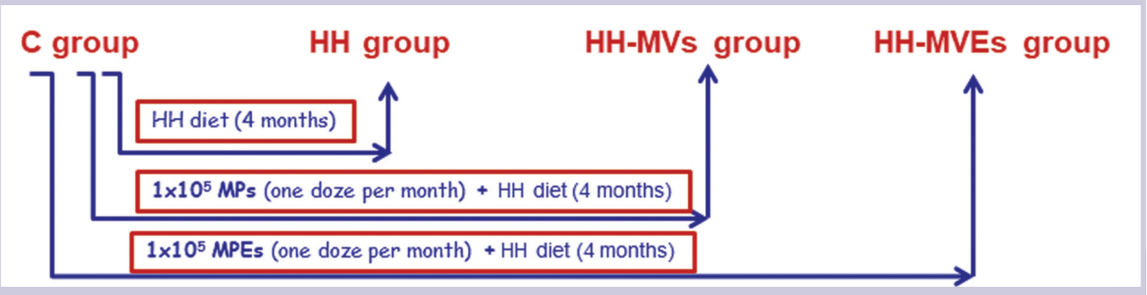
Background: The development of atherosclerosis and cardiovascular disease is the result of multiple intermediate processes where endothelial dysfunction and vascular inflammation play key contributing roles. Cell-derived microparticles or microvesicles (MPs or MVs), endothelial progenitor cells (EPCs) and circulating microRNAs (miRNAs) have attracted major interest as biomarkers and potential regulators for atherosclerotic vascular disease, but their involvement in the mechanisms of inflammatory processes and vascular repair remain controversial.

We aimed to: (1) investigate the potential beneficial effects of circulating microparticles (MPs) of healthy origins on EPC dysfunctionality in atherosclerosis as well as involved mechanisms; **(2)** evaluate the biological activity and functional role of MPs, in particular of the EPCs-derived MPs (MPEs), of healthy origins in reducing atherosclerotic vascular disease development.



In vitro experimental design for the late EPCs

*The late EPCs were obtained and expanded in culture from the peripheral blood mononuclear cells (PB-MNC) isolated from two animal groups: hypertensive-hyperlipidemic hamsters **(HH),** and control hamsters **(C)***



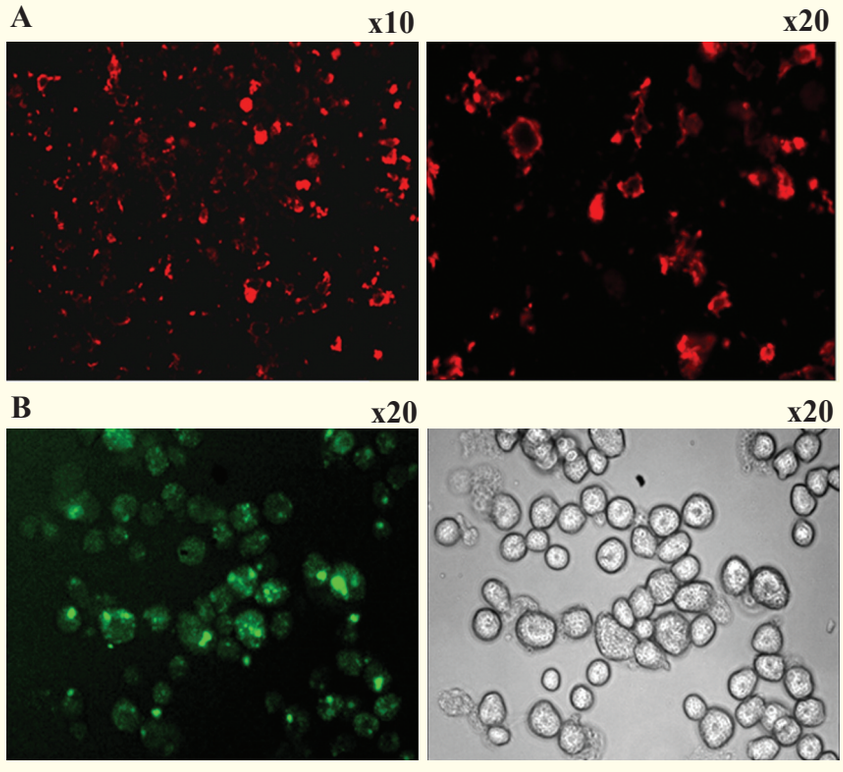
In vivo experimental design for hamster groups

HH group** = **simultaneously hypertensive-hyperlipidemic -** by combining two feeding conditions: the standard chow supplemented with 3% cholesterol and 15% butter, for hyperlipemia, and 8% NaCl diet, for hypertension, for 4 months, to induce **atherosclerosis

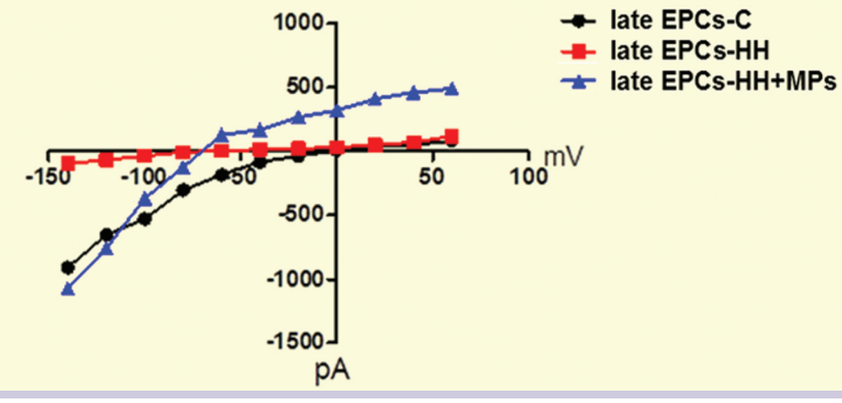
***HH-MPs/HH-MPEs groups =** HH with retro-orbital sinus injection containing **1x10⁵ MPs or EPC-derived MPs (MPEs),** from control hamster, one doze per month, for 4 months of HH diet, to prevent atherosclerosis*

***C = controls,** age-matched normal healthy animals kept in the same housing conditions and receiving a normal chow diet containing basal 1% NaCl*

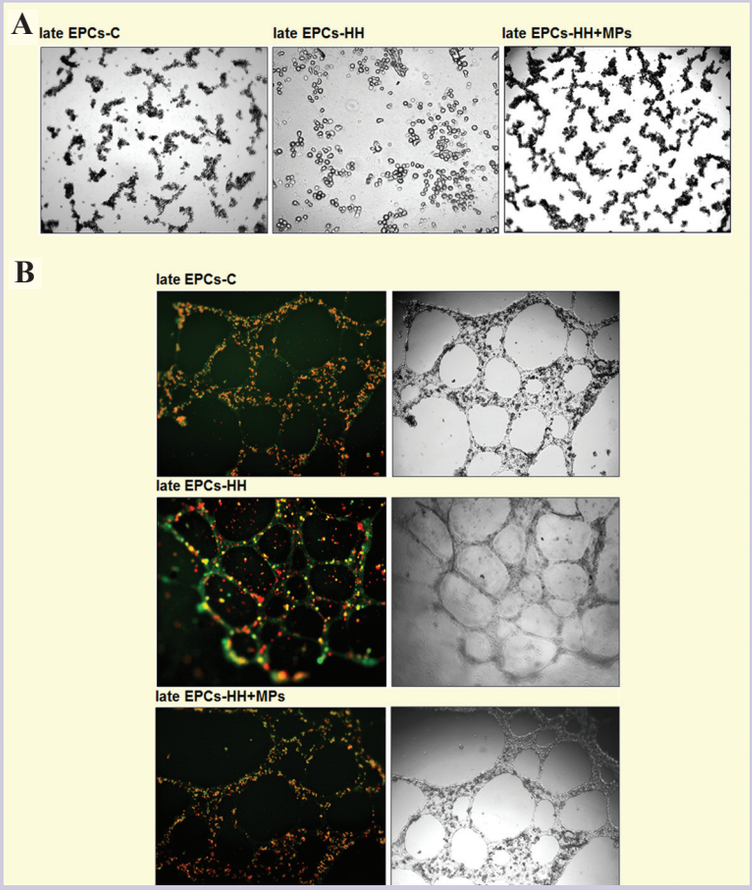
Results in vitro: The results showed that late EPCs display endothelial cell phenotype: **(1)** have ability to uptake Dil-Ac-LDL and UEA-1; **(2)** express CD34, CD133, KDR, CD144, vWF, Tie-2. Late EPCs from HH exhibited different morphological and functional characteristics compared to control: **(1)** are smaller and irregular in shape; **(2)** present decreased endothelial surface marker expression; **(3)** display reduced proliferation, migration and adhesion; **(4)** lose ability to organize themselves into tubular structures and integrate into vascular network; **(5)** have diminished function of inward rectifier potassium channels. The incubation of late EPCs with MPs improved EPC functionality by miR-10a, miR-21, miR-126, miR-146a, miR-223 transfer and IGF-1 expression activation; the kinetic study of MP incorporation into EPCs demonstrated MP uptake by EPCs followed by the miRNA transfer.



*In vitro cellular uptake of PKH26-labeled MPs **(A)** and siRNA-FITC transfected MPs **(B)** by the fluorescence microscopy. For these experiments the late EPC-HH were incubated with MPs of healthy origin.*



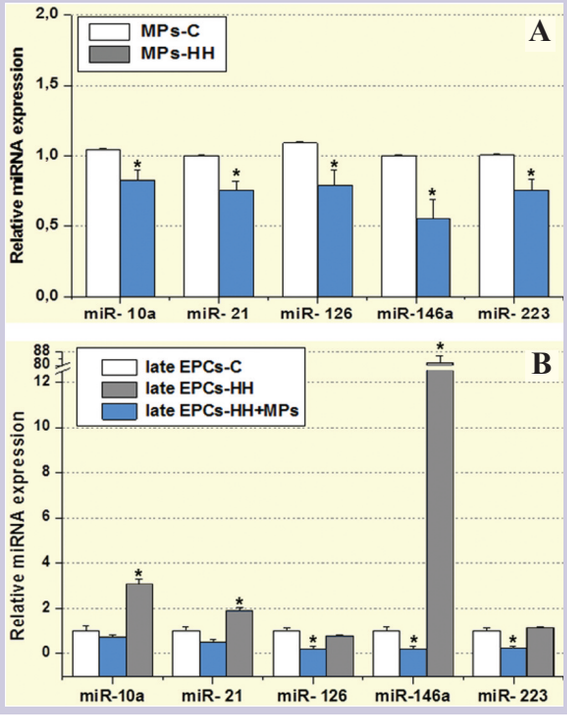
The I-V relationship plotted from average currents recorded by patch-clamp technique sing step pulses from -140 to +60mV during 400-500ms for late EPCs-C, late EPCs-HH and late EPCs-HH+MPs.



(A) In vitro angiogenesis assay; representative images for capillary-like tube formation onto Matrigel for late EPCs-C, late EPCs-HH and late EPCs-HH+MPs. The imagines were taken using the phase contrast microscopy (the original magnification was ×5).

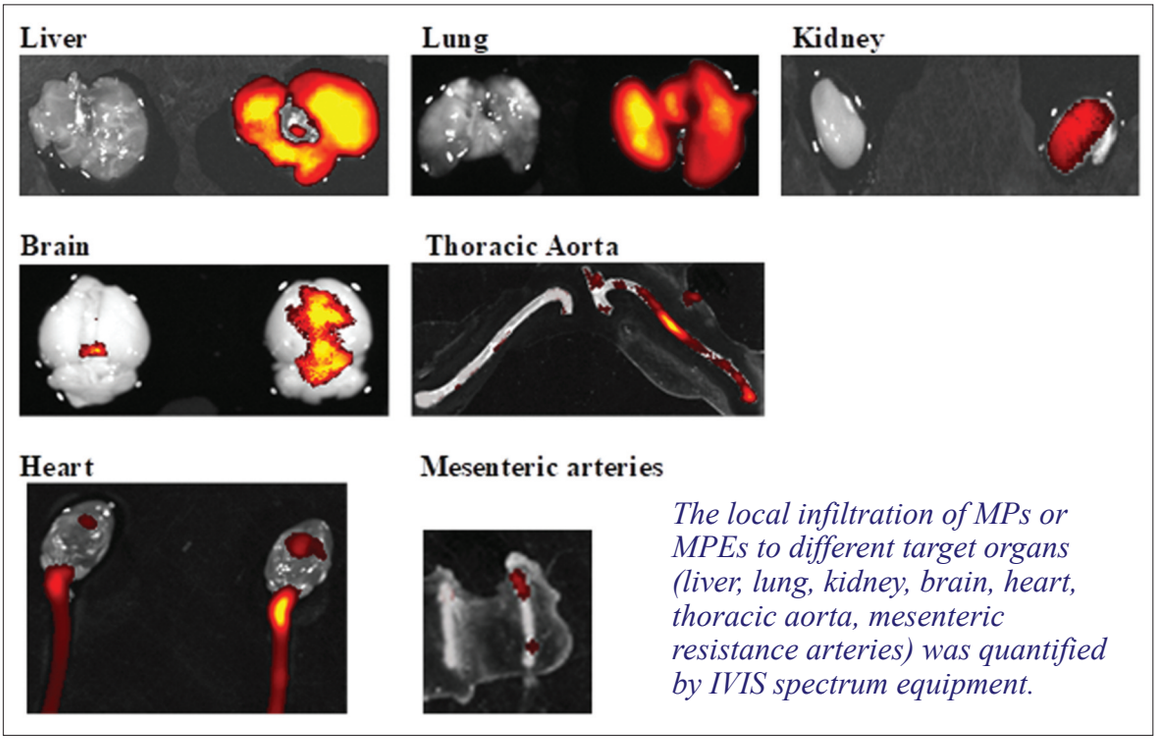
(B) In vitro vasculogenesis assay; representative images for the incorporation of late EPCs-C, late EPCs-HH and late EPCs-HH+MPs labeled with Dil-Ac-LDL (red) into capillary-like tubes by HUVECs v

The evaluation of miRNA expression in: ***(A)*** *MPs from C and HH groups, and* ***(B)*** *late EPCs-C, late EPCs-HH and late EPCs-HH+MPs cultures, by qRT-PCR; the relative quantification of miR-10a, miR-21, miR-126, miR-146a, miR-223.*

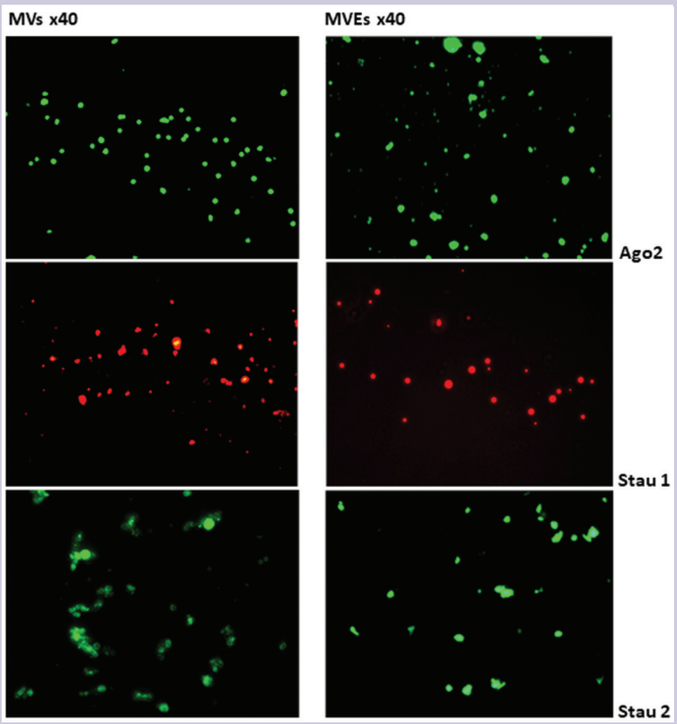


Conclusion: The data reveal that late EPCs from atherosclerotic model exhibit distinctive features and are dysfunctional, and their function recovery can be supported by MP ability to transfer miRNAs. ***(N Alexandru et al., 2017, Acta Physiologica, - awarded by UEFISCDI)***

Results in vivo: We found that circulating MP/MPE transplantation significantly suppresses the development of atherosclerosis processes via: **(1)** the alleviation of dyslipidemia, hypertension, circulating EPC levels, cytokine/chemokine profiles (VEGF, IL-6, IL-8); **(2)** the structural and functional remodelling within the vessel wall and heart in term of: distensibility/stiffness/pulse wave velocity of thoracic aorta, carotid wall thickness, systolic and diastolic function of left ventricle, left ventricular hypertrophy, lipid accumulation/contraction/relaxation in thoracic aorta, carotid and resistance arteries. We also demonstrated that: **(1)** circulating MPs operate as protective and delivery system for miRNAs in circulation – this was demonstrated by validating MPs/MPEs as intercellular carriers of functional Ago2-miRNA, Stau1-miRNA and Stau2-miRNA complexes; **(2)** MPs and MPEs significantly protect against atherosclerotic vascular disease via transfer of miR-10a, miR-21, miR-126, miR-146a to circulating late EPCs. It mentioned that, the favorable effects of MPEs are greater than those of MPs.

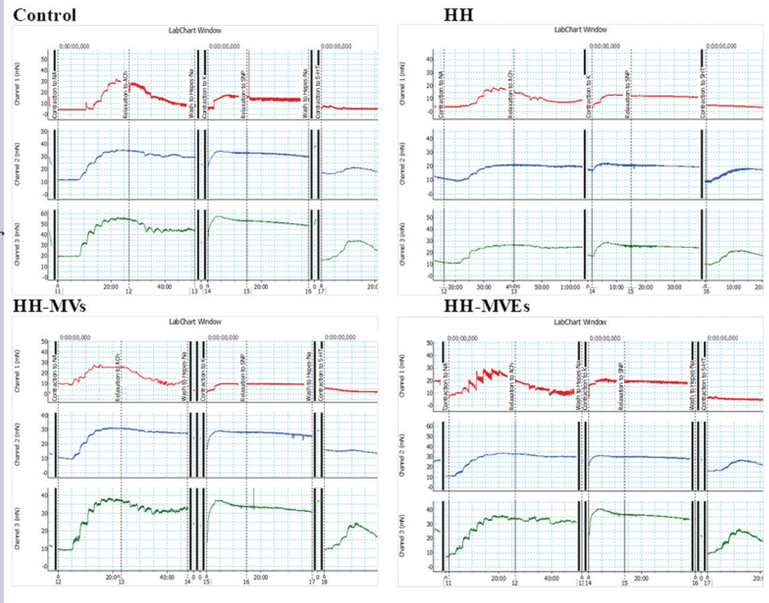


The local infiltration of MPs or MPEs to different target organs (liver, lung, kidney, brain, heart, thoracic aorta, mesenteric resistance arteries) was quantified by IVIS spectrum equipment.



The appearance of fluorescence suggests that MPs/MPEs shuttle ribonucleoproteins (Ago2, Stau 1, Stau 2) bound to miRNA and involved in its traffic.

The MP/MPE administration to atherosclerotic hamster model reduced the development of vascular dysfunction due to hyper-lipemic - hypertensive diet: This was showed by the functional investigation of vascular wall using myograph technique: contraction (to NA, 5-HT, K+) and relaxation (to ACh, SNP) in resistance arteries (up), carotid arteries (middle), and thoracic aorta (down) explanted from C, HH, HH-MPs, HH-MPEs hamsters.



Conclusion: In addition to our in vitro study, the vivo study indicates that transplanted MPs and MPEs of healthy origins to an atherosclerotic animal model (HH hamsters), can counteract hypertensive-hyperlipidemic diet induced detrimental effects on plasma, structural and functional parameters by biologically active miR-10a, miR-21, miR-126, miR-146a transfer to circulating EPCs mediating their vascular repair function in atherosclerosis processes. ***(N. Alexandru, A Constantin et al., 2019, under review)***

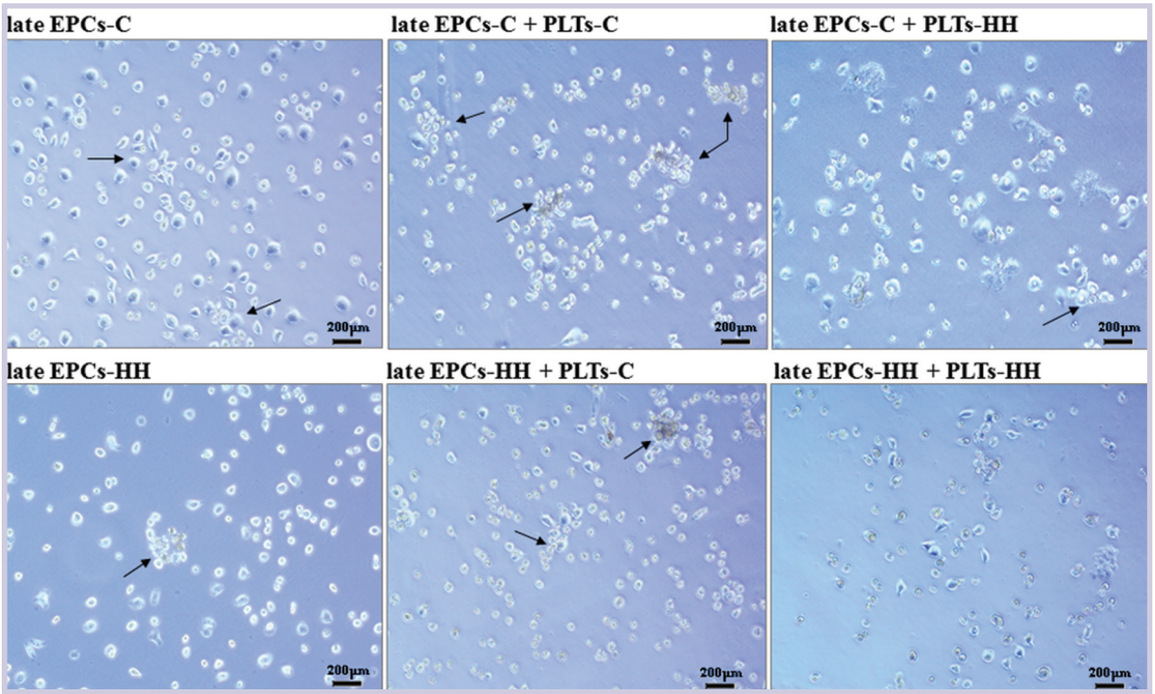
2. NEW INSIGHTS IN PLATELET-ENDOTHELIAL PROGENITOR CELL INTERPLAY IN ATHEROSCLEROTIC DISEASE

The purpose was to evaluate the effect of platelets on functional properties of late endothelial progenitor cells (EPCs), in the direct co-culture conditions, and to investigate the involved mediators, in experimental induced atherosclerosis.

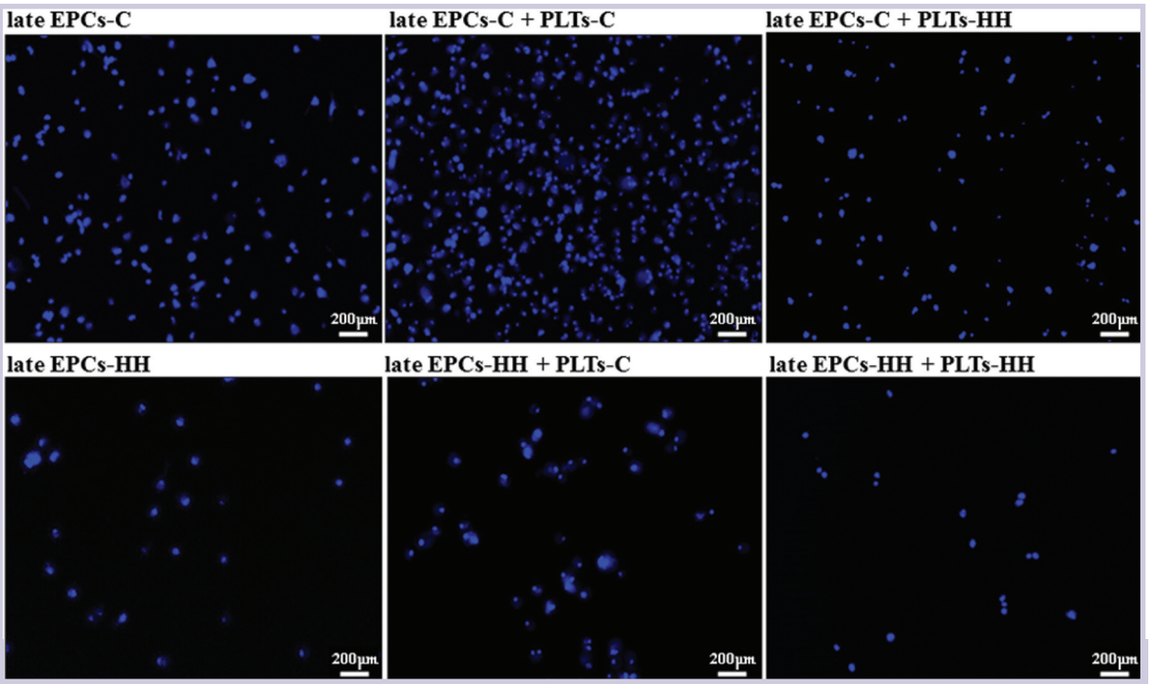
In vitro experimental design

The late EPCs obtained from two animal groups, hypertensive-hyperlipidemic (HH) and control (C) hamsters, named late EPCs-HH and late EPCs-C, were co-incubated with or without platelets isolated from both groups.

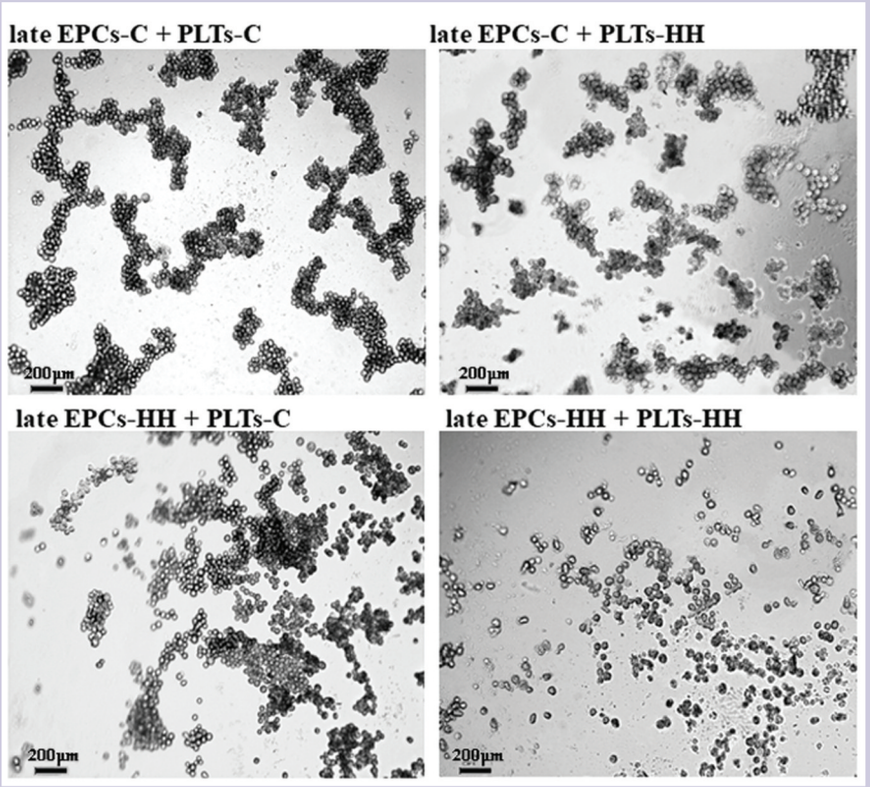
Results: Our results have showed that exposure to platelets from control animals: **(I)** promoted the late EPCs-C capacity to form colonies and capillary-like structures, and also to proliferate and migrate; **(II)** improved the functional properties of late EPCs-HH; **(III)** strengthened the direct binding EPCs-platelets; **(IV)** increased SDF-1,VEGF, PDGF and reduced CD40L, IL-1β,-6,-8 levels; and **(V)** enhanced miR-223 and IGF-1R expressions. Platelets from HH group diminished functional abilities for both EPC types and had opposite effects on these pro-angiogenic and pro-inflammatory molecules. Furthermore, testing the direct effect of miR-223 and IGF-1R on late EPCs disclosed that these molecular factors improve late EPC functional properties in atherosclerosis in terms of stimulation of the proliferation and migration abilities.



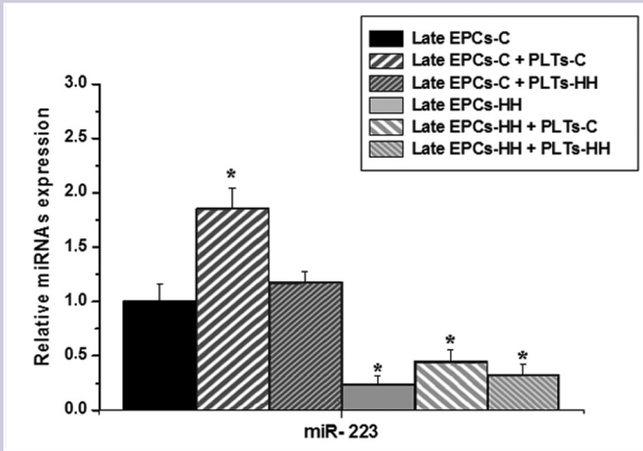
Representative EPC colonies after 7 days of co-incubation with isolated platelets from C (PLTs-C) and HH (PLTs-HH) groups, compared with EPCs alone (on collagen type I)



Representative images with migrated late EPCs-C, late-EPCs-HH stained with DAPI and incubated with or without PLTs-C or PLTs-HH.



Representative images for capillary-like sprouts designed by late EPCs-C+PLTs-C, late EPCs-C+PLTs-HH, late EPCs-HH+PLTs-C and late EPCs-HH+PLTs-HH onto Matrigel.



The analysis of miR-223 expression in cultures of late EPCs-C or late EPCs-HH co-incubated with or without platelets from C group (PLTs-C) or platelets from HH group (PLTs-HH) by RT-qPCR.

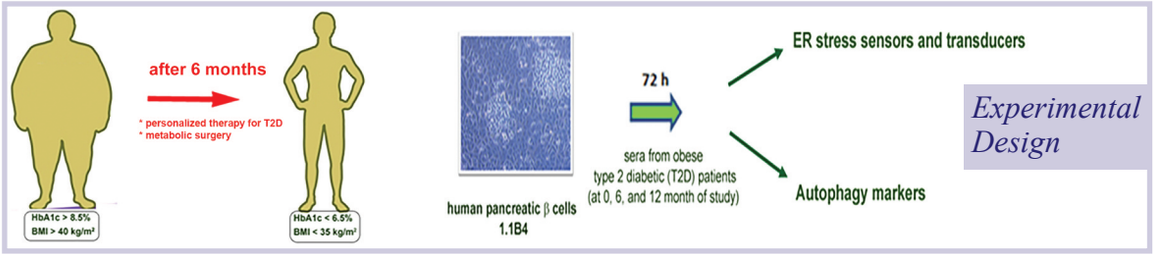
Conclusion:

In vitro exposure to platelets of healthy origins had a positive effect on functional properties of athero-sclerotic late EPCs. The most likely candidates mediating EPC-platelet interaction can be SDF-1, VEGF, CD40L, PDGF, IL-1β,-6,-8, miR-223 and IGF-1R. The current study brings evidences that the presence of healthy origin platelets is of utmost importance on functional improvement of EPCs in atherosclerosis. ***(N. Alexandru et al., 2019, Frontiers in Pharmacology / Inflammation Pharmacology)***

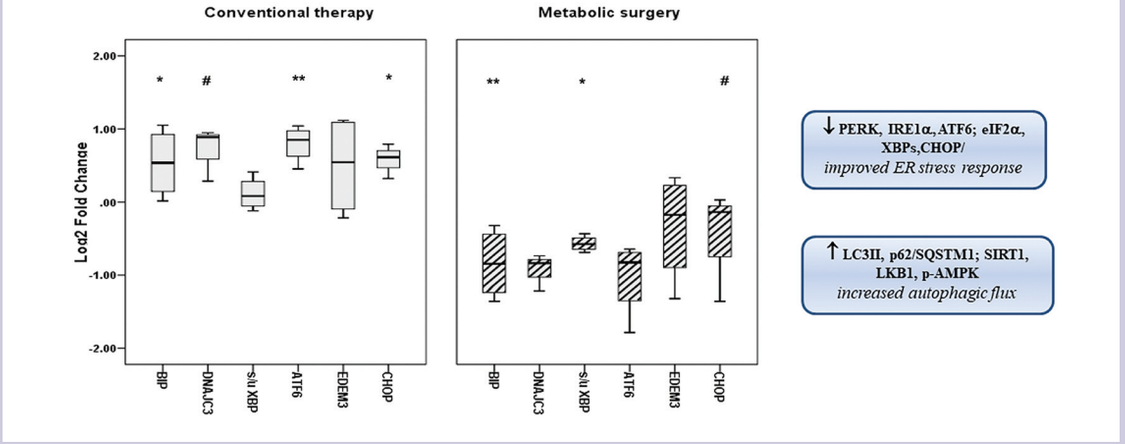
3. DEVELOPMENT OF A SELECTION PROTOCOL FOR METABOLIC SURGERY FOLLOWING THE EVALUATION OF ITS IMPACT ON THE REMISSION OF TYPE 2 DIABETES IN PATIENTS WITH OBESITY

Project Coordinator: Regina Maria Hospital, Bucharest
Partner: ICBP Nicolae Simionescu

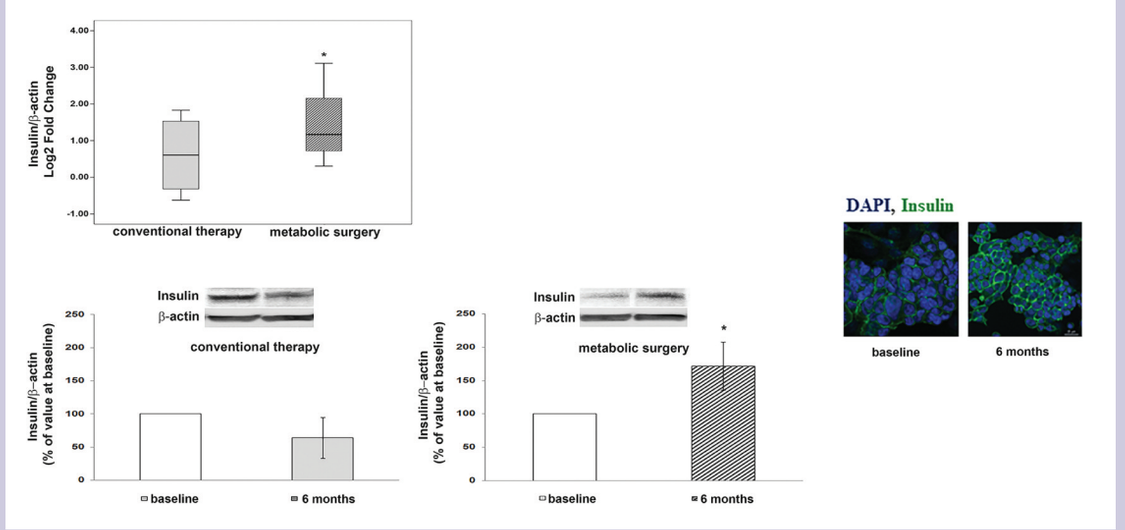
We aimed at investigating cellular responses of human beta cells exposed to sera from obese type 2 diabetic (T2D) patients treated differently, namely by conventional therapy or laparoscopic sleeve gastrectomy (LSG).



Results: At 6-months follow up, patients undergoing LSG achieved an adequate glycaemic control, whereas conventionally treated patients did not. As compared to 1.1B4 cells incubated with baseline sera (control), cells exposed to sera from LSG-treated participants exhibited: **(i)** increased viability and proliferation ($p<0.05$); **(ii)** diminished levels of ROS and p53 ($p<0.05$)**; (iii)** enhanced protein expression of autophagy-related SIRT1 and p62/SQSTM1 ($p<0.05$); **(iv)** significantly decreased transcript levels of endoplasmic reticulum (ER) stress markers ($p<0.05$); and **(v)** augmented insulin expression ($p<0.05$). Conversely, the 6 months conventional therapy appeared not to impact on circulating redox status. Moreover, 1.1B4 cells exposed to sera from conventionally treated patients experienced mild ER stress.



Sera of patients reaching an adequate glycemic control in 6 month post-metabolic surgery contained factors with beneficial effects for beta cell survival.



Insulin protein and mRNA levels in 1.1B4 beta cells exposed to sera from patients who underwent LSG 6 months ago were significantly increased

Conclusion: Circulating factors in sera from patients with improved diabetes after metabolic surgery exerted favourable effects on beta cell function and survival. ***(A. Constantin et al., 2019, Obesity Surgery)***

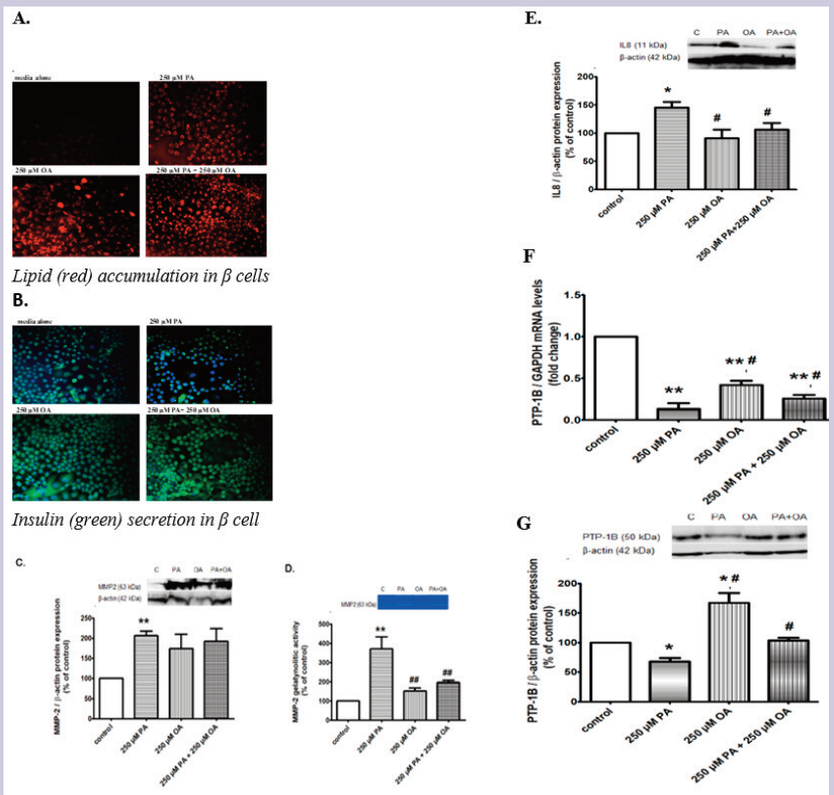
Another purpose of this project was to identify the mechanisms underlying the different effects of palmitic acid and oleic acid on human pancreatic beta cell function. To address this problem, the oxidative stress, endoplasmic reticulum stress, inflammation, apoptosis and their mediator molecules have been investigated in the insulin releasing beta cells exposed to palmitic and/or oleic acid (PA/OA).

Experimental Design

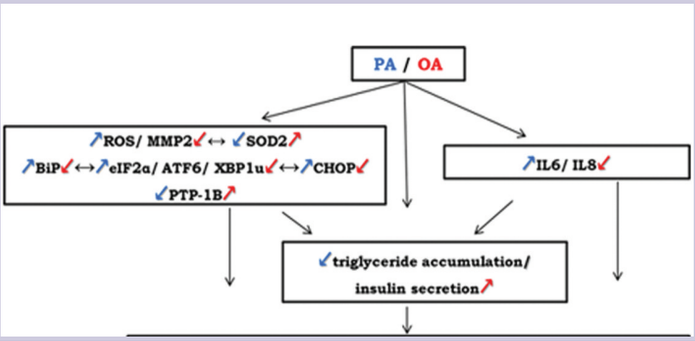
- *cell viability/proliferation (MTT)*
- *neutral lipid accumulation (Fluorescence Microscopy)*
- *insulin secretion (Fluorescence Microscopy, Elisa Method)*
- *gene expression (RT-PCR)*
- *protein expression (**Western** Blot)*
- *intracellular ROS production (DCFH)*
- *gelatinolytic activity (Zymography)*

Results:

In cultured 1.1B4 beta cells OA promotes neutral lipid accumulation and insulin secretion, PA is poorly incorporated into triglycerides and does not stimulate insulin secretion from human pancreatic islets at physiologically glucose concentrations. In addition, PA caused: **(1)** oxidative stress through increases in ROS production and MMP-2 protein expression/gelatinolytic activity associated with down-regulation of SOD2 protein; **(2)** endoplasmic reticulum stress by up-regulation of chaperone BiP protein and UPR transcription factors (eIF2α, ATF6, XBP1u proteins) and by PTP-1B down-regulation in both mRNA and protein levels; **(3)** inflammation through enhanced synthesis of proinflammatory cytokines (IL6, IL8 proteins); and **(4)** apoptosis by enforced proteic expression of CHOP multifunctional transcription factor. OA alone had opposite effects by reduction of the ROS levels and MMP-2 activity, down-regulation of BiP, eIF2α, ATF6, XBP1u, CHOP, IL6, IL8 and by SOD2 and PTP-1B overexpression. The supplementation of saturated PA with the monounsaturated OA reversed the negative effects of PA alone.



Co-supplementation of 250 μM palmitic acid (PA) with 250 μM oleic acid (OA) reversed the effects of PA alone regulating insulin secretion from pancreatic β cells through ROS, MMP-2, ATF6, XBP1u, IL8 reduction and SOD2, PTP-1B activation. These effects may be associated with an increase in insulin secretion and accumulation of neutral lipids in β cells leading to β cell regulation by OA treatment.



A potential mechanism of OA regulating human β cell survival and function by linking oxidative stress with endoplasmic reticulum stress, apoptosis and inflammation within the β cells.

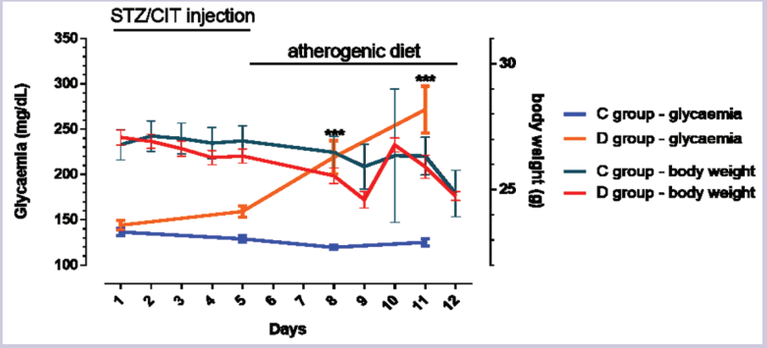
Conclusion:

Oleic acid has a protective action against palmitic acid on beta cell lipotoxicity through promotion of triglyceride accumulation and insulin secretion and regulation of some effector molecules involved in oxidative stress, endoplasmic reticulum stress, inflammation and apoptosis. (***M. Nemecz, et al., 2019, Frontiers in Pharmacology / Ethnopharmacology)***

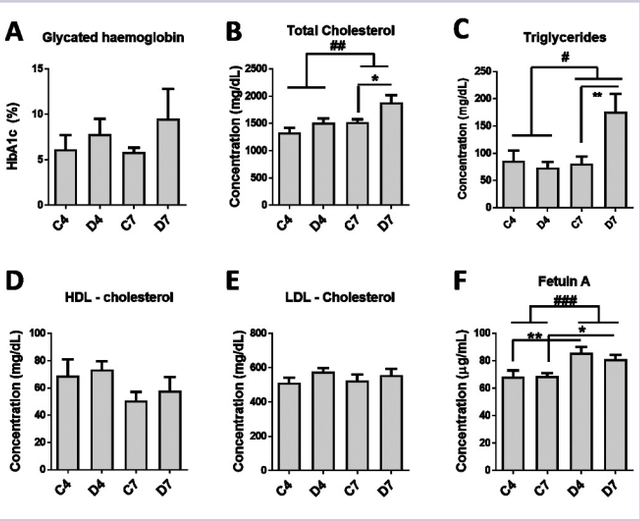
4. ESTABLISHING CORRELATIONS BETWEEN STRUCTURAL AND FUNCTIONAL CARDIAC CHANGES AND PLASMA AND HEMODYNAMIC PARAMETERS IN ATHEROSCLEROSIS-ASSOCIATED DIABETES MELLITUS

Background: Diabetes appears to contribute directly to the development of cardiovascular disorders including heart valve disease. Aortic valve disease and especially calcific aortic valve disease is a global health burden in all aging societies, including the Romanian population. There is currently no drug therapy available for a dysfunctional heart valve, the only option being repair or replacement.

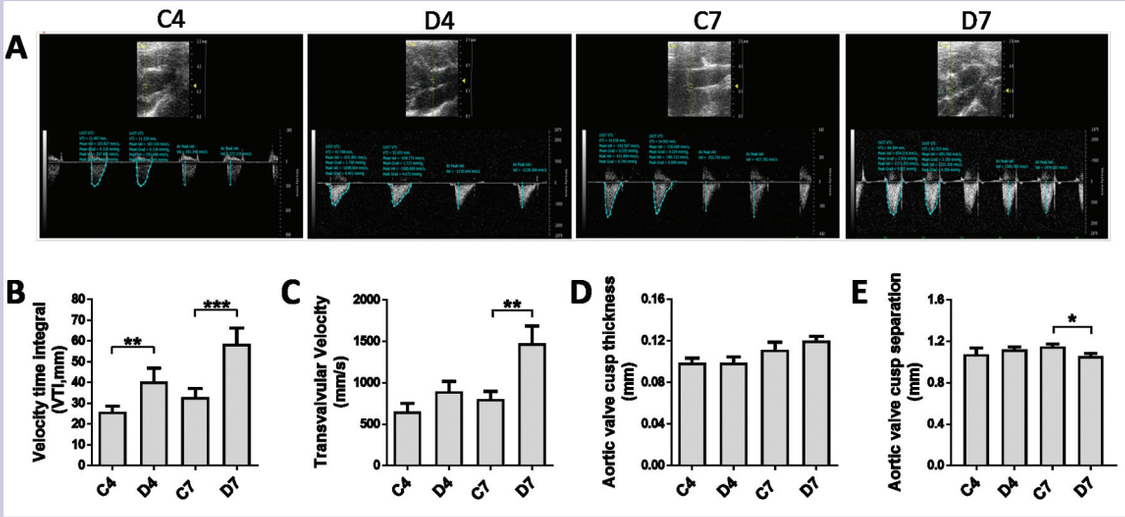
This study aimed to determine which are the structural and functional changes relevant for early stage of aortic valve disease induced by diabetes in atherosclerosis and to establish a correlation between these changes and biochemical plasmatic and hemodynamic echographic parameters relevant for diabetes state.



Graphical representation of experimental animal models. *Glycemia and body weight evolution during STZ/CIT treatment and atherogenic diet: 55 mg/kg body weight streptozotocin (STZ) or equivalent volume of citrate buffer (CIT) were administrated for five consecutive days and then standard chow was changed to atherogenic diet until the sacrifice on day 9 or 12 after the first STZ or CIT injection (or at 4 and 7 days after the last STZ/CIT injection). Four experimental groups of ApoE-/- mice were established: D4, D7 and C4, C7, representing diabetic animals (D) or control animals (C) sacrificed at 4 and 7 days, respectively, after the last streptozotocin or citrate injection.*



Plasma parameters measured in diabetic versus control experimental groups. *Levels of plasma glycated hemoglobin expressed as percent of total hemoglobin (A) total cholesterol (B), triglycerides (C), HDL cholesterol (D), LDL cholesterol (E) and Fetuin A (F), all expressed as mg/dl were measured in the STZ-treated diabetic (D) and citrate treated non-diabetic (C) groups subjected to atherogenic diet for 4 and 7 days.*



Echocardiographic comparison of aortic valve hemodynamics in hyperlipemic ApoE-/- and hyperlipemic ApoE-/- diabetic mice groups. (A) Representative echocardiographic PW Doppler Mode images for velocity time integrals and transvalvular velocity. Quantification of velocity time integrals (B), transvalvular velocity (C), cusp thickness (D), cusp separation (E).

The results showed early aortic valve dysfunction detected by echography after one week of diabetes; lesions were found in the aortic root in the first week of diabetes. Significant correlations were found between tissue valve biomarkers and plasmatic and hemodynamic parameters.

Conclusion: Our study may help to understand the mechanisms of aortic valve disease in the diabetic milieu in order to discover and validate new biomarkers of cardiovascular aortic valve disease in diabetes and reveal new possible targets for therapies. ***(M. M. Tucureanu, A. Filippi et al., 2019, Diabetes and Vascular Disease Research)***

COLLABORATION

INTERNATIONAL

• Cardiovascular Tissue Engineering and Regenerative Medicine Lab, Clemson University, USA (Prof. Dr. Dan Simionescu)

• Biocompatibility and Tissue Regeneration Lab, Clemson University, USA (Prof. Dr. Agneta Simionescu)

• Department of Medical Physics, M. Smoluchowski Institute of Physics, Jagiellonian University, Kraków, Poland (Prof. Dr. Ewa Stepien)

NATIONAL

• Internal Medicine Clinic, Emergency Clinical Hospital, Calea Floreasca Street, Bucharest, România (Conf. Dr. Elisabeta Bădila, MD)

• University of Bucharest, Faculty of Biology: Department of Anatomy, Physiology and Biophysics (Prof. Dr. Violeta Ristoiu, Conf. Dr. Dana Cucu)

• National Institute for Research and Development in the field of Pathology and Biomedical Sciences "Victor Babeş" Bucharest (Dr. CSI. Mihaela Gherghiceanu)

• University of Medicine and Pharmacy "Carol Davila" Bucharest (Prof. Dr. Dragoș Vinereanu)

• University and Emergency Hospital Bucharest, Cardiology Department, Bucharest, România (Prof. Dr. Dragoș Vinereanu)

• Emergency Clinical Hospital "Pius Brînzeu" Timişoara - Research Center OncoGen (Prof. Dr. Virgil Păunescu)

• Fundeni Clinical Institute, Bucharest (Prof. Dr. Irinel Popescu)

• "Grigore T. Popa" University of Medicine and Pharmacy, Iași (Dr. CSI, Viorel Scripcariu)

• University of Medicine and Pharmacy, Craiova (Prof. Dr. Adrian Săftoiu)

• University of Medicine and Pharmacy of Târgu Mureș, Clinical Department of Internal Medicine, Târgu Mureș, România (Dr. Dan Nistor)

• Proestetica Medical Center, Bucharest, Romania (Dr. Dana Jianu)

• Institute of Diabetes, Nutrition and Metabolic Diseases "Prof. Dr N. Paulescu", Bucharest, România (Prof. Dr. Constantin Ionescu-Tîrgoviște)

• Regina Maria Hospital, Bucharest, România (Dr. Cătălin Copăescu)

ONGOING GRANTS AWARDED BY COMPETITION (2016-2020)

• **2016-2020 - Grant of the Ministry of National Education and Scientific Research, Romania, MNE-NASRI (INTERMEDIATE BODY FOR RESEARCH): Competitiveness Operational Programme 2014-2020 Priority Axis 1 – Research, Technological Development And Innovation (Rd&I) To Support Economic Competitiveness And Business Development. Action 1.1.4** Attracting high-level personnel from abroad in order to enhance the RD capacity**. Project Title:** Targeted therapies for diabetes - related aortic valve disease. **Grant no.** 115/13.09.2016/ **Project Code:** 104362. **Project acronym:** Theravaldis**, Specialist in Implementation Project Team:** Adriana Georgescu; **Executive Manager:** Ileana Mânduțeanu; **Project Manager:** Agneta Simionescu

• **2018-2020 - Grants of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI - Complex Projects Completed in Consortia CDI (PCCDI), under Program 1. Developing national CD, Subprogram 1.2. Institutional performance - "Institutional Development Project". Project no. PN-III-P1-1.2-PCCDI-2017-0527 / Contract no. 83 PCCDI/2018 - Project title:** Development of BIOnanotechnologies based on

extracellular Vesicles for early diagnosis, prognosis and therapy of Atherosclerotic disease; **Project acronym:** BIOVEA **Project manager** Adriana Georgescu; ***Partners***: National Institute for Research and Development in the field of Pathology and Biomedical Sciences "Victor Babeş" Bucharest; University of Medicine and Pharmacy "Carol Davila" Bucharest; University and Emergency Hospital Bucharest, Cardiology Department, Bucharest; Emergency Clinical Hospital "Pius Brînzeu" Timişoara - Research Center OncoGen.

● **2018-2020 – Grants of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI - Complex Projects Completed in Consortia CDI (PCCDI), under Program 1. Developing national CD, Subprogram 1.2. Institutional performance - "Institutional Development Project" Project no. PN-III-P1-1.2-PCCDI-2017-0797/Contract no. 66 PCCDI /2018- Project Title:** Pathogenic mechanisms and personalized treatment in pancreatic cancer using multi-omic technologies. **Project acronym**: PANCNGS – **Project manager** Irinel Popescu **- Project responsible IBPC** Maya Simionescu; **Partners**: Fundeni Clinical Institute, Bucharest; "Grigore T. Popa" University of Medicine and Pharmacy, Iași; University of Medicine and Pharmacy, Craiova; University of Bucharest, Faculty of Biology: Department of Anatomy, Physiology and Biophysics.

● **2018-2020 - Grants of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI - Postdoctoral research projects (PD), under Program 1. Developing national CD, Subprogram 1.1. Human Resources**. **Project no. PN-III P1-1.1-PD-2016-1660 - Project title:** Tissue engineering of blood vessels using three-dimensional bioprinting of endothelial and smooth muscle progenitor cells; **- Project acronym:** BIOPRINT **– Mentor for the project** Adriana Georgescu

COMPLETED GRANTS AWARDED BY COMPETITION (1999-2018)

INTERNATIONAL PROJECTS

● **2001-2002 - Grant from the Deutsche Forschungsgemeinschaft (DFG).** Grant in collaboration with Institute of Pharmacy and Food Chemistry, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany. **Project:** Detection of non-enzymatic glycation products of cellular ADN using experimental models of diabetic animals - **Project manager** A. Georgescu

● **2008-2011- COST PROGRAMME, EU COST Action BM 0602. The project:** Adipose tissue cells dialogue in obesity, diabetes and inflammation; search for molecules of pharmacological potential in reducing adipose tissue inflammatory proteins. **Project manager** D. Popov

● **2012-2014 – Grant awarded by ERC starting project - UEFISCDI: ERC-like – type 'Grant Support' - Capacity project: project ID PNII-CT-ERC-2012-1"-**(Grant no. 6/18. 07. 2012*)*; **Project:** Circulating platelet microparticles and endothelial progenitor cells in vascular atherosclerosis: new pathophysiological and therapeutic implications - **Project manager** A. Georgescu

NATIONAL PROJECTS

● **1999- 2000 - Grant from Romanian Academy, –** Grant no. 31/ 1999. **Project:** The role of aging and of association for a long time of the hyperglycemia-hyperlipemia in the formation of the glycated irreversible proteins. The effect of the in vivo administration by L-arginine – **Project manager** A. Georgescu

● **1999- Grant Awarded By Romanian Research and Technology Ministry, –** Grant no. 881/ 10.09.1999. **Project:** The effect of the simultaneously hyperglycemia –hyperlipemia on the vascular reactivity of the resistance arteries in the presence of PGF2alpha vasoconstrictor. The involved cellular mechanism - **Project manager** A. Georgescu

• **1999- Grant Awarded By Romanian Research and Technology Ministry. Project**: The effect of simultaneously hyper-glycemia-hyperlipemia on the morphological and functional changes of the organs affected in hypertension - **Project manager** D. Popov

• **2000- Grant from Romanian Academy, –** Grant no. 138/ 1.10.2000. **Project:** The study of the mechanisms involved in the vascular response of the resistance arteries in the presence of bradykinine. The effect of hyperglycemia-hyperlipemia on the endothelium dependent relaxation. - **Project manager** A. Georgescu

• **2000-Grant Awarded By Romanian Research and Technology Ministry, –** Grant no. 55/ 1.12.2000. **Project:** The effect of the combined hyperglycemia –hyperlipemia on the vascular reactivity of the resistance arteries in the presence of potassium vasoconstrictor. The involved cellular mechanism. - **Project manager** A. Georgescu

• **2000-2001**. **ANSTI. Project:** A new action for clotrimazole: modulation of the vascular reactivity of the resistance arteries and of the metabolism of cells within the vascular wall. **Project manager** G. Costache

• **2001- MEC. Project:** Endothelial nitric oxide synthase gene polymorphisms as potential risk factors for developing cardiovascular complications in diabetes. **Project manager** G. Costache

• **2001 – 2002 - Grant Awarded By: Romanian Ministry of Research- National Research Program for Fundamental Research VIASAN, –** Grant no. 110/ 29.10.2001. **Project:** The effect of of enoxaparin sodium on the vascular reactivity of the resistance arteries in aging and in diabetes; the role of nitric oxide - **Project manager** A. Georgescu

• **2001 – 2002 - Grant Awarded By Romanian Research and Technology Ministry, –** Grant no. 7051/2001. **Project:** The gap junctions and the vascular reactivity of the mesenteric resistance arteries; the effect of the heptanol - **Project manager** A. Georgescu

• **2002 -** National Consortium: Institute of Diabetes, Nutrition and Metabolic Diseases "Prof. Dr N. Paulescu", "Cantacuzino" Institute and Institute of Cellular Biology and Pathology "N. Simionescu". **Project:** Effect of antioxidants on carbonyl stress modulation in diabetes. **- Project manager** A. Constantin

• **2001-2003- Grant Awarded By: Romanian Ministry of Research- National Research Program For Fundamental Research VIASAN**. The cardiovascular changes associated with type 1 diabetes at transgenic mouse model - **Project manager** D. Popov

• **2002 – 2005 - Grant Awarded By: Romanian Ministry Of Research- National Research Program For Fundamental Research VIASAN, –** Grant no. 171/ 7.10.2002. **Project**: The pharmacological properties and the cellular mechanisms involved in the effect of nebivolol in the renal artery in diabet; the experimental data - **Project manager** A. Georgescu

• **2004 – 2006 - Grant Awarded By: Romanian Ministry Of Research- National Research Program For Fundamental Research VIASAN, –** Grant no. 347/ 1.10.2004**. Project:** The effect of the enoxaparin (a low molecular weight heparin) in the reestablishment of the endothelial vascular dysfunctions in aging and in diabetes; the involvement of the mitogen-activated protein kinase evidentiated by changes in the expression of c-fos gene and transcription factor AP-1 - **Project manager** A. Georgescu

• **2004-2006 - VIASAN. Project:** Adiponectin as mediator of the insulin-activated intracellular signaling - clinical implications in obesity associated with type 2 diabetes **- Project manager** A. Constantin

• **2006-2008 - Excellence Research Projects for young researchers (CEEX) from the , -** Grant no. 15121/2006**; Project:**

The effect of elevated levels of shed membrane microparticles on the function of the peripheral veins at patients with chronic venous insufficiency - **Project manager** A. Georgescu

• **2007-2008 - CNCSIS TD - Project:** Strategies to combat cardiovascular complications in obesity: the anti-inflammatory effect of Rosiglitazone in the recovery of atheroma instability and endothelial dysfunction caused by adipokines**. - Project manager** A. Constantin

• **2008-2009: Grant from Ministry of Education, Research and Youth, CNCSIS, National Program for Research-Development and Innovation 2 (PNCDI-2), Program Human Resources/ Research Projects for young PhD. Students- TD type. Project:** The studies of platelet dysfunction associated with vascular system changes in ageing and pathological conditions; **- Project manager** N. Alexandru

• **2008-2011- National Program for Research-Development and Innovation 2 (PNCDI-2), National Centre for Programs' Management (CNMP), Partnerships Program 4, Direction 4 – Health –** Grant no. 42138/ 1.10.2008. **Project:** Ratio of circulating microparticles to endothelial progenitor cells, a new cellular marker of endothelial dysfunction induced by combined hypertension and hypercholesterolemia; anti-atherosclerotic effect of irbesartan - **Project manager** A. Georgescu

• **2008-2011- National Program for Research-Development and Innovation 2 (PNCDI-2), Ministry of Education, Research and Youth, The National Authority for Scientific Research, Idei Program 1 – Funding Application for Exploratory Research Projects –** Grant no. 1159/19.01.2009. **Project:** Vascular complications of small arteries in patients with obesity associated or not with type 2 diabetes; the endothelial dysfunction and insulin resistance - **Project manager** A. Georgescu

• **2014-2017 - PN-II-PT-PCCA-2013-4-2154. Contract no. 207/2014 – Project:** Developing a protocol for selecting patients with obesity and type 2 diabetes mellitus (T2DM) eligible for diabetes remission after metabolic surgery" (CREDOR - Collaborative Romanian Efforts for Diabetes and Obesity Retrench). **Project responsible IBPC** G. Costache

• **2015-2017: Grant of the Romanian National Authority for Scientific Research and Innovation, CNCS –UEFISCDI, Program Human Resources/ Project number PN-II-RU-TE-2014-4-0523:** New insights in platelet-endothelial progenitor cell interplay in atherosclerotic disease; **Project manager** N. Alexandru

• **2015-2017- Grant of the Romanian National Authority for Scientific Research and Innovation, CNCS –UEFISCDI, Program Human Resources/Project number PN-II-RU-TE-2014-4-0525:** *Grant no 79/01.10.2015***. Project:** Microparticles as intracellular delivery strategies for microRNAs and potential therapies for atherosclerotic vascular disease - **Project manager** A. Georgescu

AWARDS

- **"Emil Racoviță "Award** of the Romanian Academy, 1991 (Doina Popov)
- "**Nicolae Simionescu** Award of the Romanian Academy, 2008 (Adriana Georgescu)
- **"Dr. Constantin Velican"** Award of the Romanian Society for Cell Biology, 2001 (Doina Popov); 2010 (Adriana Georgescu)
- **Second Prize** at the **European Life Scientist Organization** Meeting, Nice, France, 2002 (Adriana Georgescu)
- **"Young Investigator"** Award of the Healthy Nutrition Foundation, 2002 [First Prize-Adriana Georgescu, Second Prize-Alina Constantin (Carale)]
- **"Agora Diabetologica"** Award of the Romanian Society for Diabetes, Nutrition and Metabolic Diseases, Berlin-Chemie, Menarini Group and Eli Lilly 2002 [Alina Constantin (Carale)]
- "**European Society on Vascular Biology and Medicine**" Award for paper presentation, 4th European Meeting on Vascular Biology and Medicine, Bristol, U. K., 2007 (Adriana Georgescu)
- **Developing World Scientist Grants of the International Society on Thrombosis and Haemostasis** for the presentation at XXII Congress of International Society on Thrombosis and Haemostasis, Boston – USA, 2009 (Adriana Georgescu).
- **Prize of the Award Committee** of the 3rd International Congress and 29th Annual scientific session of Romanian Society for Cell Biology**,** 2011 (Adriana Georgescu et al.)
- **Prize for Poster Presentation to "22nd World Congress of International Federation for the Surgery of Obesity and Metabolic Disorders"**, London, UK, 2017 (Alina Constantin et al.)
- **Scientific Achievements – Original Article Awards** of the Ministry for Education and Research (32 award original articles)

DEPARTMENT OF PATHOPHYSIOLOGY AND PHARMACOLOGY

Holidays in the Institute - Day of the Romanian Blouse.