DOI: 10.1111/eci.12926

ABSTRACT

PHAGOCYTE BIOLOGY

P002-T | ARHGAP25 is a crucial player in the pathomechanism of rheumatoid arthritis in mice

R. Csépányi-Kömi; R. Pusztai; T. Svanya; P. Lévay; É. Wisniewski; K. Ella; Á. Sűdy; E. Ligeti

Department of Physiology, Semmelweis University, Budapest, Hungary

Background: ARHGAP25, a GTP-ase activating protein for Rac is mainly expressed in hematopoietic cells and plays predominant role in regulation of neutrophilic effector functions (phagocytosis, superoxide production), as well as neutrophil recruitment and extravasation due to its effect on Rac-dependent cytoskeletal changes. These findings strongly suggest, that ARHGAP25 may be involved in regulation of inflammatory diseases as well. This led us to investigate its role in autoantibody-induced model of rheumatoid arthritis, in transgenic mice.

Material and methods: After intraperitoneal injection of K/B×N mouse strain-derived serum into Arhgap25$^{-/-}$ (KO) and wild type (WT) mice, ankle thickness was measured and a clinical score, indicating the severity of inflammation was determined. The loss of function was investigated by hanging on the mice on a wire-grid. Neutrophil infiltration into the ankle joints and the amount of filamentary actin in infiltrated neutrophils were measured with flow cytometry, using KO or WT mice.

Results: Absence of ARHGAP25 caused a remarkable decrease in clinical scores, as well as in ankle thickness compared to wild type mice. Similar results were observed in the functional test: ARHGAP25$^{-/-}$ animals spent longer time on the grid. Moreover, lack of ARHGAP25 significantly reduced the infiltrated neutrophil count in ankle joints and F-actin amount in infiltrated neutrophils compared to WT. In mice treated with control serum neither inflammation, nor loss of function or elevated neutrophil infiltration could be observed, regardless of the genotype.

Conclusions: Our results suggest, that lack of ARHGAP25 result in the reduction of the signs of inflammation, as well as the neutrophil count in arthritic ankle joints, which may be linked to the altered actin-reorganization. Our current results indicate, that beyond the elementary phagocyte functions, ARHGAP25 is a crucial player of inflammation in a human disease-related complex model.

This work was supported by OTKA K108382 grant.

P003-T | Altered homeostasis of peripheral neutrophils correlates with Alzheimer's disease progression

Y. Dong*; J. Lagarde†; L. Xicota‡; H. Corne†; Y. Chantran*; T. Chaigneau*; M. Bottlaender§; M.-C. Potier‡; P. Aucouturier*; G. Dorothée*; M. Sarazin†; C. Elbim*

**Inserm UMR-S 938, CdR Saint-Antoine, team "Immune System, Neuroinflammation and Neurodegenerative Diseases", Paris, France; †Unit of Neurology of Memory and Language, Centre Hospitalier Sainte Anne, Paris, France; ‡Institut du Cerveau et de la Moelle épinière, ICM, Paris, France; §UNIACT, NeuroSpin, Institut d'Imagerie Biomédicale, Direction des sciences du vivant, Commissariat à l'Energie Atomique, Gif-sur-Yvette, France*

Background: Alzheimer's disease, the most common form of dementia in the elderly, is characterized by a triad of pathological features: extracellular amyloid deposits predominantly composed of amyloid-β peptides, intracellular neurofibrillary tangles made of hyper- and abnormally phosphorylated Tau protein, and gliosis. Recent studies have underlined the effect of systemic inflammation on the pathophysiology of Alzheimer's disease. Neutrophils are key components of early innate immunity and contribute to uncontrolled systemic inflammation if not tightly regulated. The aim of our study was to fully characterize human circulating neutrophils at different disease stages in Alzheimer's disease.

Material and methods: We analyzed neutrophil phenotypes and functions in forty-two patients with Alzheimer's disease (sixteen with mild cognitive impairment and twenty-six with dementia), and compared them with twenty-two age-matched healthy subjects. This study was performed directly in whole blood to avoid issues with data interpretation related to cell isolation procedures.

Results: Blood samples from Alzheimer's diseases patients with dementia revealed neutrophil hyperactivation associated with increased reactive oxygen species production and increased levels of intravascular neutrophil extravascular traps. The homeostasis of circulating neutrophils in these patients also changed: the ratio between the harmful hyperreactive CXCR4 high/CD62L low senescent and the CD16 bright/CD62L dim immunosuppressive neutrophil subsets rose in the later stage of the disease. Interestingly, these abnormalities were greater in fast-decliner than in slow-decliner patients.

Conclusions: Our results demonstrate that the homeostasis of peripheral neutrophils is altered in Alzheimer's disease patients, with a shift towards enhanced proinflammatory properties. By modifying the systemic inflammatory equilibrium and potentially helping to weaken the blood brain barrier, this altered neutrophil homeostasis may help to drive disease progression. Most important, our data strongly suggest that the neutrophil phenotype may be associated with the rate of cognitive decline and may thus constitute an innovative and prognostic blood biomarker in patients with Alzheimer's disease.

P004-T | The role of myeloperoxidase in the regulation of polymorphonuclear neutrophil cell death

L. Kubala*; S. Kremserova†; A. Kocurkova‡; M. Chorvatova§; A. Klinke¶

International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic; †Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic; ‡Department of Biochemistry, Faculty of Science MU, Brno, Czech Republic, Brno, Czech Republic; §Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic, Brno, Czech Republic; ¶Heart Center, University of Cologne, Cologne, Czech Republic

Polymorphonuclear neutrophils (PMNs) are important participants in host defense. However, if they stay at the site of injury for a long time, they can cause chronic inflammation. Thus the removal of PMN from the site of injury is an essential process which is mediated by programmed cell death. It is supposed that myeloperoxidase (MPO), highly abundant enzyme in PMN granules, primarily connected with PMN defense machinery participates in regulated cell death of PMNs. But mechanisms and pathways how MPO influences PMN cell death have not been revealed yet.

PMNs were isolated from bronchoalveolar lavage of wild-type and MPO-deficient mice with induced acute lung inflammation by lipopolysaccharide. PMNs were activated by PMA, opsonized Streptococcus mutans, and fmlp. Phosphatidylserine externalization (Annexin V/propidium iodide assay) was determined together with caspase 3 activity using Ac-DEVD-AMC. Reactive oxygen species were determined by luminol-enhanced chemiluminescence. DNA fragmentation was determined together with levels of selected proteins. Under selected conditions PMNs were incubated with MPO inhibitor 4-ABAH, pan caspase inhibitor Z-VAD-FMK, a protease inhibitor pepstatin A, and an inhibitor of neutrophil elastase.

MPO-deficient PMNs showed significant decrease in expression of phosphatidylserine on outer side of membrane after activation by PMA. Thus MPO-deficient PMNs revealed declined rate of programmed cell death. However, PMA treated PMNs do not show activation of other typical markers of apoptosis, like DNA fragmentation, activation of pro-apoptotic proteins BID and BAX and activation of caspase 3 and 8. On the other hand presence of autophagic markers was noticed in stimulated cells, particularly cleavage of LC3 protein and increased expression of protein p62.

In conclusion, except production of HOCl, the effect of MPO during inflammation can be connected to ability of MPO to regulate the life span of PMNs presented at the site of injury.

P005-T | The effects of natural pseurotins on functions of macrophages

D. Rubanova*; O. Vasicek*; J. Svenda†; L. Kubala‡

Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic; †Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic; ‡International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Natural pseurotins are secondary metabolites of filamentous fungi possessing antimicrobial and antiparasitic activity. Interestingly a few studies suggested effects of pseurotins in eukaryotes e.g. antiangiogenic activity in chick chorioallantoic membrane assay or inhibition of IgE production by activated B-lymphocytes. In this study, we focused on effects of natural pseurotins and pseurotin structural analogs on physiological functions of macrophages as main myeloid cell populations in the body.

The murine peritoneal macrophages (RAW264.7) were stimulated by Escherichia coli lipopolysaccharide. The effect of natural pseurotins A and D and some pseurotin structural analogs on the production of nitric oxide (NO) and on the expression of inducible nitric oxide synthase in macrophages was studied. The influence on the production of pro-inflammatory cytokines was also measured.

The cytotoxic effect of pseurotins was evaluated and it was found that they were not cytotoxic to macrophages. Pseurotins and their analogs decreased the production of NO and the expression of inducible nitric oxide synthase by activated macrophages in a concentration-dependent manner. They have also a reducing effect on the production of pro-inflammatory cytokines, except for the one of analogs.

In conclusion, natural pseurotins and their analogs attenuate the response of macrophages on the stimulation of bacterial lipopolysaccharide.

The study was supported by the GACR of the Czech Republic (17-18858S).

P006-T | Conditionally immortalised HoxB8 mouse cells recapitulate adhesion-dependent stimulation of primary neutrophils

J. Chu; B. McCormick; G. Mazelyte; S. Vermeren
The University of Edinburgh, Edinburgh, United Kingdom

Background: Neutrophils are peripheral leukocytes that represent the first line of defence against infections. They are also important regulators of the inflammatory response. Neutrophils are terminally differentiated, short-lived cells that cannot be cultured, transfected or transduced. They are therefore frequently studied in mouse models or in neutrophil-like cell lines such as PLB-985. However, neutrophil-like cells lines are imperfect models that do not always recapitulate events that occur in primary neutrophils, as exemplified by adhesion-dependent events. Here we show that neutrophils derived from conditionally immortalised HoxB8 mouse bone marrow progenitor cells are morphologically, functionally and mechanistically comparable with primary neutrophils, presenting a useful experimental model.

Materials and Methods: Mouse bone marrow derived hematopoietic progenitor cells were conditionally immortalised by transduction with ER-Hoxb8 retrovirus as described. Signalling events and neutrophil functions were analysed in in vitro differentiated HoxB8 neutrophils, primary (mouse/human) neutrophils and differentiated PLB-985.

Results: Compared with differentiated PLB-985 cells, HoxB8 neutrophils had a morphology that more resembled that of primary neutrophils. HoxB8 neutrophils were responsive to integrin and FcγR as well as to GPCR stimulation with regards to signalling events induced and in a range of functional assays, whereas PLB-985 neutrophils were only responsive to GPCR stimulation. HoxB8 neutrophils expressed integrin and FcγRs to a similar extent as primary neutrophils, whereas the PLB-985 neutrophils lacked CD16, a major FcγR. Ectopic expression of CD16 was not sufficient to render differentiated PLB-985 responsive to integrin and/or FcγR stimulation.

Conclusions: The HoxB8 neutrophils appear to be a reliable experimental model system for mechanistic studies in primary neutrophils.

P007-T | Characterization of neutrophils generated in vitro from Hoxb8-transduced myeloid progenitor cells

A. Orosz; A. Mócsai
Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary

Background: Acute inflammation and neutrophil granulocytes have long been mentioned together. However due to the lack of effective in vitro models, we are still unaware of most of the molecular mechanisms that conjoin them. We are hoping to overcome this obstacle using ex vivo generated neutrophil cells from the so called SCF ER-Hoxb8 progenitors. These bone marrow-derived progenitors are retrovirally transduced to express the Hoxb8 transcription factor in the presence of β-estrogen, in order to keep them in progenitor state for long periods of time. From them, unlimited amounts of neutrophils can be differentiated using certain cytokines.

Materials and methods: The SCF ER-Hoxb8 progenitor cells were cultured in medium supplemented with SCF and β-estradiol. Neutrophils were grown in β-estrogen free medium supplemented with G-CSF. Cell surface markers were detected using flow cytometry. ROS production was measured according to cytochrome-c reduction. Adhesion and migration capabilities were tested using different stimulating agents. Phagocyting properties were measured using USA 300 GFP expressing Staphylococcus aureus.

Results: $CD45^+$ progenitor cell line can be cultured for long time. $CD11b^+$ and $Ly6G^+$ neutrophils start to differentiate in 4 days. 5–6 days old neutrophils show strong adhesion upon PMA, TNFα and IC stimulation, however migration and ROS production is more pronounced with IC activation than with TNFα. These effector functions deteriorate with time, and the cells usually die on the 8–9th day after β-estrogen removal. They can also phagocyte 60–70% of opsonized bacteria.

Conclusions: The SCF ER-Hoxb8 cell line can be a great alternative to the common-genetically modified mice-based methods in use to discover the molecular basis of the role of the neutrophils in inflammatory diseases. The whole system is now ready to be used and culturing protocols provide great number of cells to carry out experiments with both in vitro and in vivo.

P008-T | Systemic development of non-specific inflammation after spinal cord injury

M. Baltin; E. Yamalitdinova; T. Baltina
Kazan Federal University, Kazan, Russian Federation

Spinal cord injury (SCI) causes a pathophysiological processes, such as a local ischemia and inflammation. In the focus of inflammation, the main function of polymorphonuclear leukocytes is the phagocytosis of dying cells. The active oxygen radicals generated by neutrophils and other phagocytic cells are an important protective mechanism of inflammatory processes that underlie nonspecific immunity. The aim of the work was to study of the phagocytic activity of polymorphonuclear leukocytes of peripheral blood in rats with SCI using local hypothermia as a neuroprotective effect. The non-linear laboratory rats were divided into two groups. The SCI was performing at the level of Th8-9 by the A. Allen's method. The first group did not receive the further treatment; the second group received procedure of local hypothermia for 20 minutes after SCI. The blood sampling was collected prior to SCI and on 1, 7, 14, 21, 30 days after SCI from the caudal vein. The blood smears were stained by method of Romanovsky – Giemsa, the counting of leukocytes was made by the "meander method". The activation of the inflammatory reaction in the early stages of SCI was characterized by a decrease in a number of the neutrophils in the first group (31·2 ± 2·746%, $P < 0·05$) as compared with intact animals (40 ± 1·8). A reliable change in a number of basophils and eosinophils in both groups was not found. A significant increase of neutrophils in the second group was observed on the 7th, 14th and 30th days compared with the first group. In the acute phase of SCI it was not found the differences in a number of neutrophils after hypothermia treatment, which provides a theoretical basis for using of local hypothermia as a neuroprotective therapy for ischemia and inflammation in the early stages of SCI.

Funded by RFBR, research project № 17-04-01746A

P010-T | Peripheral phagocyte characteristics as markers of systemic inflammation in rats with different stages of Parkinson's disease

M. Rudyk*; I. Hurmach*; V. Svyatetska*; O. Prysyazhnyuk*; T. Dovbynchuk*; O. Skachkova†; G. Tolstanova*; L. Skivka*
**Taras Shevchenko National University of Kyiv, Kyiv, Ukraine; †National Cancer Institute, Kyiv, Ukraine*

Background: Local inflammation caused by activated microglia is implicated in the pathogenesis of Parkinson's disease (PD). Progression of this neurodegenerative disease is characterized by the development of systemic inflammation with the involvement of phagocytes from different locations. The aim of our study was to evaluate metabolic and phenotypic markers of different phagocyte populations in rats with 6-OHDA-induced PD with mild and severe stages of disease.

Material and methods: Parkinson-like neurodegenerative lesion was induced in Wistar rats by a single unilateral stereotaxic injection of 6-Hydroxydopamine (6-OHDA) into the striatum. Apomorphine-induced rotation test was used to assay the extent of neuronal loss. Flow cytometry was used to analyze ROS production, phagocytosis activity as well as expression of CD14, CD80/86 and CD206 by microglia, circulation and peritoneal phagocytes in rats. Arginase activity and NO production by resident microglia and peritoneal macrophages were examined in colorimetric tests.

Results: At the 29th day after 6-OHDA injection, microglia from rats with PD showed proinflammatory metabolic shift that was more pronounced in rats with severe stage of disease (with 100% loss of substantia nigra dopaminergic neurons). Sharply decreased phagocytosis activity along with increased ROS and NO production by brain phagocytes were detected in PD rats. Phenotypic profile of microglial cell was characterized by decreased expression of CD14 and CD80/86 along with high CD206 expression. Both mild and severe stages of PD were associated with pro-inflammatory activation of circulating phagocytes. Progression of the neuronal damage led to progressive monocytosis and proinflammatory activation of peritoneal macrophages.

Conclusions: Correlation between stage-dependent changes of microglia metabolism and peripheral phagocytes activity allow us to regard metabolic indices of circulating immune cells as perspective sensitive markers for early diagnostics of PD and effective monitoring of inflammatory process in brain.

P011-T | Development of a fluorescence-based osteoclast fusion assay

A. Erdélyi*; D. Csete*; E. Simon*; Á. Pánczél*; S.P. Nagy*; G. Bordács†; K. Csorba†; Z. Jakus‡; A. Mócsai*
**Department of Physiology, Semmelweis University School of Medicine and MTA-SE "Lendület" Inflammation Physiology Research Group, Budapest, Hungary; †Department of Automation and Applied Informatics, University of Technology and Economics, Budapest, Hungary; ‡Department of Physiology, Semmelweis University School of Medicine and MTA-SE "Lendület" Lymphatic Physiology Research Group, Budapest, Hungary*

Background: Osteoclasts are phagocytic cells capable of degrading bone tissue. They develop from myeloid progenitors via biochemical differentiation followed by

intercellular fusion. During the biochemical maturation several factors are expressed and activated, committing the cells towards the osteoclast fate. During fusion, mononuclear cells fuse into multinuclear cells that can perform bone resorption. Osteoclasts can be differentiated in vitro from bone marrow cells cultured in the presence of M-CSF and RANKL. Such cultures are usually stained histochemically for the osteoclast-specific TRAP enzyme. Osteoclasts are then defined as TRAP-positive cells containing at least three nuclei. Despite its wide use there are limitations of this approach. Therefore, our aim was to develop and characterize a novel, fluorescence-based assay for the identification of osteoclasts in in vitro cultures.

Materials and methods: We have used the mTmG and the mG mice for these studies. mTmG mice express a membrane-targeted tdTomato protein, whereas mG mice (generated from mTmG using a germline Cre transgene) express membrane-targeted EGFP. We cultured mTmG, mG or mixed mTmG+mG bone marrow cells towards osteoclasts. We evaluated osteoclast growth using ChemoColor, a new software dedicated for automatized high throughput image analysis.

Results: Bone marrow cells of mTmG and mG mice remained red and green, respectively. However, culturing mixed mTmG+mG cells gave rise to large "yellow" cells carrying both red and green fluorescence signals. This indicated the fusion of mTmG and mG cells. The mixed mTmG+mG osteoclast cultures showed normal kinetics of osteoclast development, and could be re-stained with TRAP. Importantly, the evaluation and quantification of the mTmG+mG cultures with ChemoColor required significantly less time than that of the TRAP-stained cultures.

Conclusions: We have set up a dual fluorescence-based fusion system that allows analysis of intercellular fusion in vitro, even under microscopy, overcoming the limitations of TRAP-staining.

P012-T | Low doses of LPS exacerbate the inflammatory response and trigger death on TLR3-primed human monocytes

M. Monguió-Tortajada*,†,‡; M. Franquesa*,§; M.-R. Sarrias¶,**; F.E. Borràs*,‡,§

**REMAR-IVECAT Group Health Science Research Institute Germans Trias i Pujol, Badalona, Spain; †ICREC Research Program Health Science Research Institute Germans Trias i Pujol, Badalona, Spain; ‡Department of Cell Biology Physiology and Immunology, UAB, Barcelona, Spain; §Nephrology Service Germans Trias i Pujol University Hospital, Badalona, Spain; ¶nnate Immunity Group Health Sciences Research Institute Germans Trias i Pujol, Badalona, Spain; **Network for Biomedical Research in Hepatic and Digestive Diseases (CIBERehd), Madrid, Spain*

Background: Monocytes can dynamically adapt to different TLR agonists inducing different patterns of inflammatory response, and the sequence of exposure to TLRs can dramatically modulate cell activation. Understanding the interactions between TLR signalling that lead to synergy, priming and tolerance to TLR agonists may help explain how prior infections and inflammatory conditioning can regulate the innate immune response to subsequent infections. Our goal was to investigate the role of MyD88-independent/dependent TLR priming on modulating the monocyte response to minimal LPS exposure.

Material and methods: Human blood monocytes were primed with agonists for TLR4 (LPS), TLR3 (poly(I:C)) and TLR7/8 (R848), and subsequently challenged to low doses of endotoxin to analyse their activation phenotype and cytokine response. Cell viability was analysed and cell death pathways studied using specific inhibitors.

Results: The different TLR agonists promoted distinct inflammatory signatures in monocytes. Upon subsequent LPS challenge, LPS and R848-primed monocytes did not enhance the previous response, whereas poly(I:C)-primed monocytes exhibited a significant inflammatory response concomitant with a sharp reduction on cell viability. Our results show that TLR3-primed monocytes are prompted to cell death by apoptosis in the presence of low endotoxin levels, concurrent with the production of high levels of TNFα and IL6. Of note, blocking of TNFR I/II in those monocytes did reduce TNFα production but did not abrogate cell death. Instead, direct signalling through TLR4 was responsible of such effect.

Conclusions: Collectively, our study provides new insights on the effects of cross-priming and synergism between TLR3 and TLR4, identifying the selective induction of apoptosis as a strategy for TLR-mediated host innate response.

P013-T | CD47-SIRPα checkpoint blockade involves kindlin-3-dependent enhancement of CD11b/CD18-integrin affinity and cytotoxic synapse formation

P. Bouti*; H. Matlung*; M. van Houdt*; P. Verkuijlen*; K. Franke*; T.W. Kuijpers*,†; T.K. van den Berg*,‡

**Sanquin Research and Landsteiner Laboratory, Amsterdam, The Netherlands; †Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands; ‡VU medical center, Amsterdam, The Netherlands*

Recently, we established that neutrophils kill antibody-opsonized tumour cells by a novel cytotoxic process that we have termed trogocytosis. This previously unknown killing mechanism involves trogocytosis (from Greek trogo, gnaw),

where fragments of target cell membrane are actively taken up by the neutrophil and this contributes to the destruction of cancer target cells. Trogocytosis and subsequent killing is strictly dependent on antibody-opsonization of the tumour cells, neutrophil Fcγ-receptor signalling and CD11b/CD18 integrin-dependent cytotoxic synapse formation. Furthermore, it is promoted by CD47-SIRPα checkpoint inhibition. Here, we present evidence that CD47-SIRPα interactions act by controlling the initial stage of the killing process i.e. the CD11b/CD18-dependent cytotoxic synapse formation. In particular, CD47-SIRPα interactions negatively regulated the CD11b/CD18 inside-out activation that occurred as a consequence of Fc-receptor signalling in neutrophils. Moreover, the inhibitory effect acted via the integrin-associated protein kindlin-3, as demonstrated, amongst other things, by using neutrophils from rare LAD-III patients that have mutations in FERMT3 and lack kindlin-3 expression. Collectively, these findings demonstrate that CD47-SIRPα interactions control a kindlin-3-dependent pathway of CD11b/CD18-integrin activation, and that targeting the CD47-SIRPα checkpoint primarily improves integrin activation, and therefore also the resultant cytotoxic synapse formation, trogocytosis and killing during neutrophil ADCC towards cancer cells.

P014-T | Sodium stibogluconate in conjunction with CD47-SIRPα checkpoint blockade enables Rituximab-mediated killing of B lymphoma cells by neutrophils

D.V. Rees*; M. van Houdt*; A. Tool*; P. Verkuijlen*; K. Schornagel*; K. Franke*; T. Kuijpers*,†; T. van den Berg*,‡; H. Matlung*

Sanquin Research and Landsteiner Laboratory, Amsterdam, The Netherlands; †Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands; ‡VU Medical Center, Amsterdam, The Netherlands

Rituximab (Rmab) is used as a first-line treatment for CD20-expressing B cell malignancies. It is believed to act by a combination of direct and immune-mediated effects, including complement- and immune cell- dependent mechanisms. However, neutrophils, the most abundant effector cells mediating antibody-dependent cellular cytotoxicity (ADCC) are incapable of killing Rmab-opsonized B lymphoma cells. Instead, Rmab triggers neutrophil trogocytosis of CD20-containing plasma membrane fragments of the target cells, which is believed to render the tumor cells resistant against further Rmab-dependent destruction. Interestingly, we found previously that neutrophils exert an entirely novel type of cytotoxicity against antibody-opsonized solid cancer cells, designated trogocytosis, which actually involves a trogocytic process. Here, we demonstrate that the lack of cytotoxicity of neutrophils towards Rmab-opsonized B lymphoblastoid cells is not due to an inherent defect of neutrophils to destroy such target cells, but rather to intrinsic properties of the CD20-Rmab complex. Furthermore, we present evidence that a combination of CD47-SIRPα checkpoint blockade and the tyrosine phosphatase inhibitor sodium stibogluconate (SSG) are able to overcome the neutrophil defect to effectively kill B lymphoma cells in the presence of Rmab. This provides opportunities for improving the clinical efficacy of Rmab treatment in cancer.

P015-T | IFNβ promotes neutrophil apoptosis and engulfment by macrophages during the resolution of inflammation

S. Schif; S. Soboh; S.K. Satyanarayanan; S. Butenko; A. kassis; N. Peled; J. Saadi; D. Barkan; A. Ariel

Department of Human Biology, University of Haifa, Haifa, Israel

The apoptosis of inflammatory neutrophils and their consequent engulfment (efferocytosis) by phagocytes during the resolution of inflammation is essential for the restoration of tissue homeostasis and prevention of autoimmunity, and results in macrophage reprogramming/immune-silencing. Here, we show that the levels of the pleiotropic cytokine IFNβ increase during the onset and resolution of murine peritonitis. In addition, we found using IFNβ$^{-/-}$ mice that this cytokine limited the onset of neutrophilic inflammation by reducing peritoneal neutrophil numbers and enhancing their apoptosis. Moreover, IFNβ enhanced the uptake of apoptotic cells in vivo and ex vivo and promoted secretion of anti-inflammatory cytokines while reducing the secretion of pro-inflammatory ones by resolution phase macrophages, thereby enhancing their reprogramming. Altogether, our results indicate IFNβ is an important mediator in the resolution of inflammation primarily by regulating macrophage efferocytosis and its consequences.

P016-T | D6/ACKR2 limits skin fibrosis: a potential role for IFNb

S. Butenko*; T. Shefer*; E. Sabo†; S. Schif-Zuck*; A. Ariel*

Department of Human Biology, University of Haifa, Haifa, Israel; †Department of Pathology, Rambam Medical Center, Haifa, Israel

Background: A successful resolution of inflammation is essential for proper wound healing and limits tissue repair short of fibrotic scarring. This process is accompanied by

tissue and macrophage reprogramming culminating in reduced interstitial levels of inflammatory mediators and elevated levels of anti-inflammatory cytokines. We have previously shown that the atypical chemokine receptor D6/ACKR2 is important for macrophage reprogramming and egress from healing tissues to the lymphatics.

Materials and methods: Skin fibrosis was induced by sub-cutaneous injection of bleomycin to WT and $D6^{-/-}$ mice. Skin fibrosis was assessed by histological analysis following H&E and Trichrome staining. In addition, enzymatically-dissociated skin sections were analyzed for cytokine and chemokine levels using ELISA, WB and qPCR.

Results: Here, we found fibrotic skin lesions from D6-deficient mice presented increased epidermal and dermal thickening, larger collagen deposition, loss of hair follicles and thinning of the subcutaneous adipose tissue. In addition, skin sections from D6-deficient mice contained increased levels of the pro-inflammatory chemokines CCL2 and 3, and reduced levels of the pro-resolving and anti-fibrotic cytokine IL-10. Interestingly, IFNβ, a newly identified pro-resolving cytokine, showed reduced levels in D6-deficient lesions at the protein and mRNA levels.

Conclusions: These findings indicate that D6 is an important effector molecule in containing inflammatory skin fibrosis and highlight the potential pro-resolving role of IFNβ in limiting tissue inflammation and its consequences.

P017-T | HLA haplotypes frequency in bone marrow donors from the Republic of Tatarstan, Russia

A. Rizvanov*; E. Kabwe*; K. Kletenkov*; S. Khaiboullina*,†; Y. Davidyuk*

**Kazan Federal University, Kazan, Russian Federation; †University of Nevada, Reno, Reno, USA*

Background: HLA genotyping is required for the bone marrow donor selection before transplantation of hematopoietic stem cells. Kazan Federal University in collaboration with one of the biggest (in Russian Federation) charity «Rusfond» is establishing non-profit organization «Kazan (Volga Region) registry of bone marrow donors» of potential bone marrow donors in Russian Federation.

Materials and methods: Blood samples from 483 potential bone marrow donors were collected in the Republic of Tatarstan (RT) and used for DNA extraction («PROTRANS DNA Box 500» kit (PROTRANS, Germany)). HLA-genotype was analyzed using SSP-PCR Cyclerplate System «PROTRANS HLA-A*/B*/Cw*» and «PROTRANS HLA-DRB1*/DQB1*» according to the manufacturer's protocols. Allele, genotype and haplotype frequencies were calculated and verified using "Arlequin ver 3.5" software.

Results: Five HLA-loci genotypes (HLA-A, -B, -C, -DRB1, and -DQB1) were examined, where the number of each allele families was as follows: 18 - A*, 27 - B*, 14 - C*, 13 - DRB1* and 5 - DQB1*. In addition, the frequency of haplotypes was investigated. Among four different two-locus haplotypes, the most frequent were HLA-A*02-C*07 haplotypes (6·94%), HLA-C*04-B*35 (11·44%), HLA-B*07-DRB1*15 (5·57%), HLA-DRB1*15-DQB1*06 (13·04%). For the two different three-locus haplotypes, the highest frequency were HLA-A*03-C*04-B*35 (5·03%) and A*03-B*35-DRB1*01 (3·33%), and for the five-locus haplotypes the most frequent was A*03-C*04-B*35-DRB1*01-DQB1*05 (3·19%). These haplotype profiles correlate with previously shown within the Russian and Tatar ethnic groups in Chelyabinsk Region of Russia and ethnic groups from European countries.

Conclusion: Collected data suggest the mixed ethnic origin of RT population. Also, HLA profiling can be used to estimate the distribution of specific alleles and haplotypes associated with diseases.

This study was supported by the Russian Government Program of Competitive Growth of Kazan Federal University and «Rusfond». AR was supported by state assignment 20.5175.2017/6.7 of the Ministry of Education and Science of Russian Federation.

P018-T | Characterizing the role of lymphatics in autoimmune arthritis

P. Aradi; Z. Horváth; É. Kemecsei; Z. Jakus

Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary

Background: Rheumatoid arthritis is the most common, systemic autoimmune disease, which is characterized by chronic inflammation of the synovial joints often resulting in progressive joint destruction and disability. The tight cooperation between the immune system and the lymphatic system is well documented in antimicrobial functions but the possible role of lymphatics in the development of autoimmune inflammatory diseases remains unclear.

Material and methods: In our studies $Flt4^{kd/+}$ mice carrying a germline point mutant kinase dead Vegfr3 allele were used. Autoimmune arthritis was induced in the K/B×N serum-transfer arthritis model, and the disease progression was followed by the measurement of ankle thickness and assessment of clinical score. The ankles were also processed for paraffin-based histology followed by

Hematoxylin and Eosin and immunostaining against lymphatic and immune cell markers.

Results: In our experiments we found the complete lack of lymphatics in the skin including the ear and joint area of $Flt4^{kd/+}$ mice, while the lymphatic structures were present in the internal organs (small intestine and lung). We characterized the development of the autoimmune arthritis in $Flt4^{+/+}$ and $Flt4^{kd/+}$ animals in the K/B×N serum-transfer arthritis model, the inflammation was reduced in $Flt4^{kd/+}$ mice. Autoimmune arthritis induction resulted in dynamically changing lymphatic morphology and unexpectedly induced lymphatic growth in $Flt4^{kd/+}$ animals with dilated lymphatic vessels.

Conclusions: Our results revealed that dynamic changes of lymphatic morphology occur in autoimmune arthritis, and the inflammation is reduced in the $Flt4^{kd/+}$ mice lacking lymphatics in the joint area. They also suggest that distinct mechanisms regulate the developmental and inflammatory lymphangiogenic program. Our findings define novel aspects of the interactions between the immune system and lymphatic structures which may stimulate the development of novel therapeutic approaches in the future for the treatment of autoimmune diseases.

P019-T | 7-ketocholesterol influences macrophage phagocytosis modulated by CPT1a

P. Calle

Instituto de Investigaciones Biomédicas de Barcelona, Barcelona, Spain

Background: Cpt1a gene encodes an enzyme involved in fatty acid oxidation. The potential of Cpt1a modulating macrophage phagocytic function has not been studied. The purpose of the study is to know if intracellular accumulation of 7-ketocholesterol (7-KC) cholesterol modulates macrophage phagocytic function through CPT1 expression.

Methods: Macrophage cell line Raw 264.7 and primary mouse peritoneal macrophages were incubated with 10 g/mL of 7-KC for 48 hours. Both macrophage cell types were transfected with short interfering RNA targeted against CPT1a and the uptake of zymosan particles was measured fluorometrically (100 zymosan green particles/hour). We evaluated lipid content by Oil Red-O stain (ORO), expression levels of CPT1a and CD36 by qPCR and phagocytic activity by zymosan uptake.

Results: Macrophages silenced for CPT1a expression, provokes a decrease in phagocytosis. Macrophage treated with 7-KC presented positive ORO stain distributed throughout the cytosol. This lipid accumulation was associated to the decreased phagocytic function and also with a decreased expression on CPT1a and CD36 mRNA levels in contrast to untreated macrophages. Finally, in macrophages silenced for CPT1a and exposed 7-KC the deficit in phagocytosis was exacerbated.

Conclusion: Our data indicate that intracellular 7-KC alter macrophage phagocytic function through CPT1a expression. The therapeutic implication of CPT1a in phagocytosis is an attractive approach to treat diseases with high macrophage involvement, such as kidney fibrosis.

P020-T | A 17 kDa fragment of lactoferrin associates with the termination of inflammation and peptides within promote resolution

S. Soboh*; S. Schif-Zuck*; A. Lutaty*; O. Zeituni-Timor*; R. Rostoker*; M.J. Podolska†; L.E. Munoz†; C. Schauer†; M. Herrmann†; A. Ariel*

**The Departments of Biology and Human Biology, University of Haifa, Haifa, Israel; †Department of Internal Medicine 3 – Rheumatology and Immunology, Friedrich-Alexander-University, Erlangen, Germany*

During the resolution of inflammation, macrophages engulf apoptotic polymorphonuclear cells (PMN) and can accumulate large numbers of their corpses. Here we report that resolution-phase-macrophages acquire the neutrophil-derived glycoprotein lactoferrin (Lf) and fragments thereof in vivo and ex vivo. During the onset and resolving phases of inflammation in murine peritonitis and bovine mastitis Lf fragments of 15 and 17 kDa occurred in various body fluids, and the murine fragmentation, accumulation and release were mediated initially by neutrophils, and later by efferocytic macrophages. The 17 kDa fragment contained two bioactive tripeptides, FKD and FKE that promoted resolution phase macrophage conversion to a pro-resolving phenotype. This resulted in a reduction in peritoneal macrophage numbers and an increase in the CD11b low subset of these cells. Moreover, FKE, but not FKD, peptides enhanced efferocytosis of apoptotic PMN, reduced TNFα and IL-6 and increased IL-10 secretion by LPS-stimulated macrophages ex vivo. In addition, FKE promoted neutrophil-mediated resolution at high concentrations (100 μM) by enhancing the formation of cytokine-scavenging aggregated NETs (tophi) at low cellular density. Thus, PMN lactoferrin is processed, acquired, and "recycled" by neutrophils and macrophages during inflammation resolution to generate fragments and peptides with paramount pro-resolving activities.

P021-T | Oxidized albumin triggers a cytokine/eicosanoid storm in leukocytes through p38 MAP kinase: role in systemic inflammation in decompensated cirrhosis

J. Alcaraz-quiles*; M. Casulleras*; K. Oettl†; E. Titos‡; R. Flores-Costa*; M. Duran-Güell*; C. López-Vicario‡; M. Pavesi§; R.E. Stauber†; V. Arroyo§; J. Clària*,‡,§

**Department of Biochemistry and Molecular Genetics, Hospital Clínic-IDIBAPS, Barcelona, Spain; †Center of Physiological Medicine, Medical University of Graz, Graz, Austria; ‡Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain; §European Foundation for the Study of Chronic Liver Failure (EF-CLIF), barcelona, Spain*

Background: Systemic inflammation is characterized by increased levels of cytokines and oxidative stress and plays a major role in decompensated cirrhosis. The endogenous factors triggering exacerbated inflammation in these patients remain unknown. The oxidized albumins, non-mercaptalbumin 1 (HNA1) and 2 (HNA2) are a common finding in cirrhosis. Both have reduced binding capacity, lack the ability to remove toxic substances and are markers of oxidative stress in cirrhosis. We examined their ability have the ability to activate innate immune cells and to act as triggers of systemic inflammation in decompensated cirrhosis.

Methods: The study included 48 healthy volunteers (HV), 41 compensated and 153 decompensated cirrhotic patients of whom 72 had ACLF. Levels and post-translational modifications of HNA1 and HNA2 were determined by HPLC and LC-qTOF/MS, respectively. Cell assays were performed in leukocytes, PBMCs and PMNs. Cytokine and eicosanoid levels in cell supernatants were measured by bead-based multiplex assays and LC-MS/MS. Gene expression was determined by RT-PCR. Kinase signaling pathways were determined by Proteome-Profiler-Human Phospho-Kinase Array and validated by western blot.

Results: Plasma HNA1 and HNA2 levels increased in parallel with the severity of the disease and correlated with markers of systemic inflammation (i.e. IL-6, IL-1β, TNF-alpha and IL-8) in patients with decompensated cirrhosis. Incubation of leukocytes and PBMCs with HNA1, at concentrations in the range detected in decompensated patients, resulted in increased IL-1 beta, IL-6 and TNF-alpha mRNA and protein expression, up-regulation of eicosanoid-generating enzymes (i.e. COX-2 and mPTGES1) and derived metabolites (PGE2, PGF2 alpha, TXB2 and LTB4). Analysis of the Phospho-Kinase Array results revealed that HNA1 actions on leukocytes were associated with phosphorylation of the p38 MAP kinase.

Conclusions: Oxidized plasma albumin triggers inflammation in peripheral immune cells, providing a rationale for the removal and replacement for freshly albumin to prevent the development of organ failures in patients with decompensated cirrhosis.

P022-T | Tissue hypoxia induces the mobilisation from the spleen of pro-angiogenic neutrophils through the activation of sympathetic nerves

C. Seignez; M. Phillipson

Department of Medical Cell Biology, Biomedical Center, Uppsala University, Uppsala, Sweden

Background: We recently described that hypoxic tissues induce the recruitment of a distinct population of neutrophils ($CD49d^+$ VEGFR1 high CXCR4 high and MMP9 high), called pro-angiogenic neutrophils, crucial for restoring the oxygen supply of the tissue by inducing the development of new and functional blood vessels[1,2]. This study aims to characterise the origin of the pro-angiogenic neutrophils and the signals driving their recruitment into the hypoxic tissue.

Materials and methods: The study is performed in a mouse model of ischemic hind limb following ligation of femoral artery. Different organs (muscles, spleen and blood) are dissociated and cell populations are analysed by flow cytometry 3 hours following ischemia induction. Different pharmacological inhibitors and surgical protocols are used to identify the molecular signals inducing the mobilisation of pro-angiogenic neutrophils visualised by histological stainings and intravital confocal microscopy.

Results: We demonstrated a substantial enrichment of pro-angiogenic neutrophils in the splenic population of neutrophils during homeostasis. Three hours following induction of ischemia in hind limb, splenic pro-angiogenic but not ordinary neutrophils are promptly mobilised into the circulation and subsequently recruited into the hypoxic muscle where they peak at two days. Intra-muscular recruitment of pro-angiogenic neutrophils is specifically blocked by the inhibition of the integrin CD49d and the receptors VEGFR1 and CXCR4. Furthermore, chemical and surgical suppression of the sympathetic signalling impaired the mobilisation of splenic pro-angiogenic neutrophils and delayed blood flow recovery of the ischemic hind limb.

Conclusions: The spleen houses a peripheral pool of the newly described pro-angiogenic neutrophils, which are quickly mobilised into the circulation by the sympathetic nerves activation and recruited into VEGF-A- and CXCL12-producing hypoxic tissues through the engagement of the integrin CD49d.

1. Massena S. et al. Blood 126(17), 2015
2. Christoffersson G. et al. Blood 120(23), 2012

P023-T | Bioengineering of the local wound environment accelerates wound healing by increasing macrophage density and induces a phenotype shift in the wound macrophages

E. Öhnstedt*; E. Vagesjö*; H. Lofton*; S. Roos†; M. Phillipson*

*Uppsala University, Uppsala, Sweden; †Swedish University of Agricultural Sciences, Uppsala, Sweden

Background: Macrophages and neutrophils are the major immune cell population of the wound and they play an important role in the healing process. In this study, we aim to accelerate wound healing by targeting the immune cells through bioengineering of the local wound microenvironment.

Material and methods: Genetically modified Lactobacillus reuteri, expressing CXCL12 (LB_CXCL12), were topically applied to full-thickness wounds on mice. Macrophages were depleted with injections of clodronate liposomes and two models of hyperglycemia were used. Wound tissue was stained for immune cell markers and mediators.

Results: Topical application of LB_CXCL12 accelerated wound healing in healthy and hyperglycemic mice, with the most prominent acceleration occurring within the first 48 hours. In the wound tissue of LB_CXCL12 treated mice there was an increased density of macrophages and proliferating dermal cells. Likewise, there was a phenotype shift where the fraction of TGF-β expressing macrophages was elevated. An increase in macrophages expressing CXCL12 could also be seen. In the macrophage depleted LB_CXCL12 treated mice, healing was decreased compared to non-depleted LB_CXCL12 treated mice.

Conclusions: Topical treatment of wounds with LB_CXCL12 accelerate wound healing in healthy and hyperglycemic mice. The accelerated wound healing is, at least partly, mediated by macrophages and the treatment induces a phenotype shift in the local wound macrophages.

P024-T | A novel drug-delivery system and drug candidate; using probiotic bacteria as bioreactors for delivery of therapeutic chemokines in wound healing

H. Lofton-Tomenius*; E. Vagesjö*; E. Öhnstedt*; A. Mortier†; S. Roos‡; M. Phillipson*

*Uppsala University, Uppsala, Sweden; †Katholieke Universiteit Leuven, Leuven, Belgium; ‡Swedish University of Agricultural Sciences, Uppsala, Sweden

Background: There is a large unmet medical need in chronic wound care. The bioavailability of therapeutic proteins and peptides administered can be subjected to an intense arsenal in vivo, especially during the wound healing process. CXCL12 is known to be upregulated during wound healing. Here we describe a method by which we have genetically modified Lactobacillus reuteri to secrete the chemokine CXCL12.

Materials and methods: The mouse and human variant of CXCL12 1a, was codon-optimized for expression in L. reuteri and inserted into an inducible expression plasmid, pSIP. The new plasmid was cloned into L. reuteri R2LC. The expression of CXCL12 relies on the peptide, SppIP for induction. AlphaLISA technology was used to detect the expression levels in bacterial cultures and freezedried product. The efficacy was tested in a mouse model of wound healing and compared to administration of recombinant CXCL12.

Results: Using this method of bio-engineered lactic acid bacteria, the recombinant CXCL12 induced levels increased up to 3-fold higher than non-induced. A synergistic effect due to the release of lactic acid by the bacteria brings a decrease in pH to the surrounding micro-environment. This lowers the activity of the CD26 protease, which without treatment, degrades the CXCL12. This results in increased bioavailability of the CXCL12. A profiling of the effect on the immune cells in the wound exhibit an increased fraction of cells expressing TGF-β, which indicates a role in the stimulation of wound healing.

Conclusion: This novel method allows for increased bioavailability of recombinant CXCL12, which alters the micro-environment around the wound, allowing for necessary cell signaling to accelerate wound healing.

P025-T | Establishing of an in vivo mouse model of epidermolysis bullosa acquisita

K. Szilveszter; T. Németh; A. Mócsai

Semmelweis University, Budapest, Hungary

Background: Investigating the pathogenesis of autoimmune diseases is important due to their severity and the lack of proper therapies. Epidermolysis bullosa acquisita is a human autoimmune skin inflammation caused by autoantibodies against type-VII collagen (C7) which is an important element of the dermal-epidermal junction. Our aim was to set up a passive in vivo murine model to study the effector phase of the autoimmune skin inflammation.

Materials and methods: Rabbits were immunized by a GST fusion protein of the autoantigenic fragment of C7. We then purified immunoglobulins from rabbit sera with column chromatography in two separate ways: with recombinant protein G agarose or Melon Gel. The purity was

checked by SDS-PAGE. In the in vivo model 12 mg IgG per mouse was injected subcutaneously on days 0, 2, 4, 6 and 8. PBS was used as control. The developing disease was followed for two weeks by measuring and scoring the affected skin area. Antibody levels against C7 in blood was tested by ELISA.

Results: The two purification methods yielded similar amounts of protein. According to Coomassie staining, the solution contained mostly immunoglobulins without any major contamination of other serum proteins. The antibody-treated mice developed a severe disease compared to the control. We observed alopecia, blisters and ulceration mostly on ears, cheek and limbs. The onset was around the 4th day from start and the severity increased through the whole investigation period. The two purification protocol resulted in similar disease course.

Conclusions: We have successfully set up in our lab an autoimmune skin blistering model. It is easily inducible and triggers a robust inflammation in a short time frame. With transgenic animals this model provides us a useful way to examine the role of specific participants in the effector phase of autoimmune diseases.

P026-T | Gorham disease – a rare disorder not easy to diagnose

L. Caba*; A. Momanu†; C.N. Gorduza‡; E.C. Popescu§; O.E. Arhire†; M.C. Panzaru*; R. Popescu*; E.V. Gorduza*

**University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania; †Rehabilitation Hospital, Iasi, Romania; ‡"St. Spiridon" Hospital, Iasi, Romania; §"Prof. Nicolae Oblu" Hospital, Iasi, Romania*

Background: Gorham's disease is a rare lymphangiomatosis characterized by progressive bone disappearance because of massive unicentric/ multicentric osteolysis. Mechanisms are still unknown. The onset is more common in teenagers or young adults.

Material and methods: Our patient is a 19-year-old, with a negative family history. The onset at 18 years was insidious with asthenia, myalgia and major bone pains, followed by a few days of sudden incomplete motor deficiency in the lower limbs and later in the upper limbs. Imaging studies (CT and MRI) reveals osteolytic lesions (C6-C7, T1-T2, first left rib, left clavicle) and pathological fracture of the C7 vertebra. Surgical procedures were: replacement of the affected vertebrae with bone grafting and prosthesis. Evolution was negative with almost complete osteolysis of the left clavicle, the emergence of new osteolysis areas in the lumbar vertebrae, bones of the pelvis and the bilateral proximal femur.

Results: The investigations performed allowed the exclusion of inflammation, thyroid/ parathyroid disease, lymphoma, neoplasia (CA 15-3, CA 19-9, CEA, CA 125 – normal), autoimmune disorders (CK-MB, rheumatoid factor, antithyroglobulin antibody, anti-dsDNA antibody, Antinuclear antibody, Circulating Normal Immune Complexes). Bone marrow biopsy shows osteolysis, replacement with connective tissue and chronic non-specific inflammation. The diagnosis was Gorham's disease. One year after the first surgical procedure, the patient presents a spastic paraplegia (with periodic recovery treatment), bilateral pyramidal syndrome, the impossibility of maintaining the sitting position, pleural effusion exudate type (recurrent with consecutive changes in the lung parenchyma). The endocrinologist recommended treatment with bisphosphonate drug.

Conclusions: Gorham disease is a multifactorial disease characterized by high morbidity and mortality and should be considered after exclusion of other pathologies with massive osteolysis. Osteolytic lesions of the spine and pleura are poor prognostic factors because of the compression of spinal nerves and the presence of chylothorax.

P027-T | Inhibition of the Arp2/3 complex in human neutrophils: comparison to the defective neutrophil functions of ARPC1B-deficient patients

E. Sprenkeler*,†; A. Tool*; M. van Houdt*; T. van den Berg*,‡; T. Kuijpers*,†

**Department of Blood Cell Research, Sanquin Research, Amsterdam, The Netherlands; †Department of Pediatric Hematology, Immunology and Infectious Diseases, Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ‡Department of Molecular Cell Biology and Immunology, VU Medical Center, Amsterdam, The Netherlands*

Introduction: The actin-related protein 2/3 (Arp2/3) complex, of importance for organizing branched actin filaments, is required for several cellular processes, i.e. cell migration and exocytosis. Recently, genetic defects in ARPC1B, a subunit of this complex, were reported. In vitro, neutrophils of these patients show defects in F-actin polymerization and neutrophil extracellular trap (NET) formation. We used CK-666, an Arp2/3 complex inhibitor, to assess if inhibition of the complex in healthy human neutrophils results in similar neutrophil defects.

Methods: Polymorphonuclear neutrophils (PMN) from healthy individuals and ARPC1B-patients were isolated from heparinized blood by centrifugation over Percoll. PMNs were left unstimulated, or pre-incubated with

CK666, and F-actin polymerization, degranulation, and NET formation was assessed.

Results: Pre-incubation of PMNs with CK-666 resulted in defective F-actin polymerization and increased neutrophil degranulation, comparable to ARPC1B-deficient PMNs. CK-666 did not aggravate the defective response of PMNs from ARPC1B-deficient patients. Although F-actin polymerization was defective, CK-666 pre-incubation did not result in an impaired NET response in healthy PMNs. Unexpectedly, upon CK-666 pre-incubation, ARPC1B-deficient PMNs were able to produce NETs.

Conclusion: Our data illustrates that inhibition of the Arp2/3 complex in PMNs of healthy individuals leads to a defective neutrophil function. Interestingly, NET formation was restored in ARPC1B-deficient PMNs upon CK-666 pre-incubation.

P028-T | Characterization of pro-angiogenic neutrophils

J. lomei

Uppsala University, Uppsala, Sweden

Background: Neutrophils are one of the most abundant white blood cells and actively take part in host immunity. Neutrophils are no longer considered to be a homogenous population and distinct sub populations with specific functions, have been identified. Recently it has been shown that a specific sub-population of neutrophils, distinguished by their expression of Cd49d, VEGFR1 and CXCR4, take active part and contribute to angiogenesis, the formation of new blood vessels.

Aim: To perform an in-depth characterization of the newly classified sub population of pro-angiogenic neutrophils (PANs), and to functionally compare them to classic neutrophils.

Material and methods: Male C57bl/6 and Flt-1 $tk^{-/-}$ deficient mice were used. PANs were compared to classic neutrophils by their size, granularity and density by using flow cytometry. In vitro phagocytosis experiment with pHredo bio particles was done. To evaluate ROS production, general oxidative stress indicator (CM-H2DCFDA) was used. In vitro angiogenesis assay, mouse aortic ring, used to evaluate pro angiogenic ability.

Results: PANs were found to be smaller in size compared to classic neutrophils and they exhibit a higher level of granularity, but the density of both populations was the same. PANs are effective phagocytes but they produced less ROS compared to the classic neutrophils. Moreover, PANs ability to induce angiogenesis was examined in an aortic ring co-culture assay. Endothelial cells grow longer sprout in presence of PANs, and the newly formed micro-vessels had a higher level of branching in presence of PANs which was partly dependent of expression of VEGFR1.

Conclusion: Our data demonstrate that PANs are a distinct sub-population of neutrophils with respect to physical feature, expression of surface markers, and functional capabilities. Combined, this shows that these cells have a different curriculum compared to classic neutrophils, resulting in a subset of neutrophils with potent pro-angiogenic properties.

P029-T | Potential use of human adipose-derived multipotent mesenchymal stromal cells as a drug delivery mechanism for bone engineering

V.V. Shupletsova*; L.S. Litvinova*; K.A. Yurova*; O.G. Khaziakhmatova*; V.V. Malashchenko*; L. Pokrovskaya†; V.L. Kudryavtseva‡; S.I. Tverdokhlebov‡; A.S. Timin‡; G.B. Sukhorukov‡,§; A.J. Gow‡,¶; E. Atochina-Vasserman‡,**; I.A. Khlusov*,†,††

**Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation; †Tomsk State University, Tomsk, Russian Federation; ‡RASA Center in Tomsk, Tomsk Polytechnic University, Tomsk, Russian Federation; §School of Engineering and Materials Science, Queen Mary University of London, London, United Kingdom; ¶Pharmacology and Toxicology, Rutgers University, Piscataway, USA; **RASA Center, Kazan Federal University, Kazan, Russian Federation; ††Siberian State Medical University, Tomsk, Russian Federation*

Background: Cell-based targeted drug delivery has potential to facilitate the incorporation and transfer of active substances to specific organs and tissues with high efficiency. Human adipose-derived multipotent mesenchymal stromal cells (hAMMSCs) represent an ideal target cell because of their ease of isolation and phagocytic function.

Material and methods: hAMMSCs were isolated from the adipose of healthy donors and characterized by surface markers. Microcapsules (2–5 μm) were made using alternated adsorption of polyelectrolytes (calcium carbonate) to generate microparticles labeled with FITC-BSA. hAMMSCs were incubated with FITC-labeled polymeric microcapsules at various ratios for 16 hours, washed and cultured for up to 190 hours. Cultured cells were analyzed for viability and phagocytosis activity using Cell-IQ v2 MLF integrated platform for a continuous real-time phase-contrast imaging. Cell media was analyzed for cytokine, chemokine levels where hAMMSCs incubated with FITC-CD90 antibodies were used as control.

Results: hAMMSCs completely phagocytosed microcapsules from the intercellular fluid in the first 16 hours, even at high concentrations (cell-to-capsule ratios of 1:46 or 1:90). Microcapsule uptake had a dose-dependent effect on hAMMSCs motor activity. The pattern of cytokine, particularly chemokine, secretion was altered by microcapsule

loading, with a decrease of IL-16, IL-18, CTACK, HGF, MCP-3, M-CSF, TRAIL and an increase of GROα, MIF and SDF-1α.

Conclusions: We demonstrated that hAMMSCs can efficiently phagocytoses microcapsules up to a ratio of 90 microcapsules per cell while maintaining their viability, secretory capability and motility up to 30 hours post phagocytosis in vitro. These findings may be a promising new strategy for hAMMSCs cells as a drug carriers across the tissue-blood or tumor-blood barrier to facilitate bone engineering or treatment of cancer. Funded: The Federal Target Program of the Ministry of Education and Science, Russian Federation (agreement 14.575.21.0164, ID number RFMEFI57517X0164)

P030-T | Viability of human polymorphonuclear leukocytes loaded with synthetic microcapsules in vitro

T. Nevzorova*; M. Nikitina*; A. Daminova*; Y. Tarakanchikova†; A. Gow‡,§; G. Sukhorukov‡,¶; R. Litvinov*,**; E. Atochina-Vasserman*,‡

**RASA Center, Kazan Federal University, Kazan, Russian Federation; †Saratov State University, Saratov, Russian Federation; ‡RASA Center in Tomsk, Tomsk Polytechnic University, Tomsk, Russian Federation; §Pharmacology and Toxicology, Rutgers University, Piscataway, USA; ¶School of Engineering and Materials Science, Queen Mary University of London, London, United Kingdom; **University of Pennsylvania, School of Medicine, Philadelphia, USA*

Background: Most potent therapeutics are unable to cross the blood-brain barrier (BBB) following systemic administration. Polymorphonuclear leukocytes (PMNLs) are good candidates as vehicles for drug delivery because of their natural recruitment across the BBB during inflammation such as in stroke or traumatic brain injury.

Material and methods: Human PMNLs were isolated from the blood of 5 healthy donors. Microcapsules (2 μm) were fabricated using alternating adsorption layers of polyelectrolytes onto calcium carbonate micro-particles. The isolated PMNLs were incubated with the FITC-microcapsules in RPMI medium at various cell/capsule ratios for 20 minutes at 37°C after which the cells were washed to remove free capsules and cultured at the same conditions. After 1, 2 or 4 hours, PMNL were analyzed for viability using flow cytometry with a MitoTracker dye. Intracellular incorporation of capsules was confirmed by transmission electron microscopy (TEM).

Results: Approximately 95% of PMNLs remained viable after 4 hours of incubation in the absence or presence of capsules. The average fraction of PMNLs loaded with microcapsules was 52%, 47%, and 48% cells at the cell/capsule ratios of 1:0·5, 1:1, and 1:1·5, respectively. The average number of viable PMNLs bearing FITC-capsules did not change significantly during the 4-hour incubation and varied from 37% to 45% irrespective of the cell/capsule ratios studied.

Conclusions: Human PMNLs can be loaded with microcapsules and maintain their viability for up to 4 hours in vitro. The incorporated microcapsules are not degraded intracellularly, which is important for the use of these capsules as drug cargos. Our findings support the potential use of natural inflammatory cells, such as PMNLs, for targeted drug delivery in neuro-inflammatory disease.

This work was supported by the Russian State project «Science», № 4.5752.2017/8.9 and by the Program for Competitive Growth at Kazan Federal University.

P031-T | Elucidating the dynamics and role of peri-vascular macrophages

K. Parv; G. Christoffersson; C.H. Hidalgo; C. Seignez; M. Phillipsson

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

Background: Macrophages are highly plastic cells specialized in sensing and responding to microenvironmental cues. Macrophages accumulate at ischemic sites where they are fundamental to tissue regeneration. The aim of the current study is to elucidate the dynamics and fate of peri-vascular macrophages following ischemic injury as well as to delineate new roles of macrophages in restoration of functional and regulated tissue perfusion.

Materials and methods: Islet transplantation into abdominal external oblique muscle and acute ischemic hind limb are used as models of ischemic tissue injury in mouse. Different transgenic mouse models are employed, including CX3CR1-GFP and Cx3CR1CreERT2; Rosa26tdTomato mice. The ischemic tissue is evaluated using intravital microscopy, immunofluorescence and flow cytometry at different time points following ischemic injury to reveal the dynamics of macrophage behavior and phenotype.

Results: Ischemic tissue injury results in rapid recruitment of monocyte-derived macrophages that attain perivascular positions along the newly formed vessels for at least two weeks. Clodronate-dependent macrophage depletion at different time points during healing is now performed to elucidate new roles of macrophages during the different phases of healing.

Conclusion: Macrophages recruited to ischemic tissue position in close contact with the newly formed vessels,

suggesting additional roles of macrophages in restitution of tissue perfusion in addition to promoting angiogenesis.

P032-T | Effect of neonatal infections on pancreatic macrophages, islet development and long-term glucose homeostasis

C.H. Hidalgo*; S. Ullsten*; E. Vagesjö*; K. Parv*; H. Liu*; A. Giraud*; P.-O. Carlsson†; M. Phillipson*

**Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden; †Department of Medical Sciences, Uppsala University, Uppsala, Sweden*

Background: The development and maturation of the islets of Langerhans in the pancreas occurs during late stages of pregnancy and continues in the neonate. After birth, insulin-producing beta cells undergo an essential process of maturation, when they amplify their capacity for glucose sensing and insulin production as well as expand in numbers.

Material and methods: Using a model of transient neonatal Staphylococcus aureus infection, we studied the effects of infections occurring at the time of beta cell maturation on islet development and long-term glucose homeostasis.

Results: Mice challenged with S. aureus during their first week of life showed impaired glucose handling, increased body weight and reduced pancreas weight (% of body weight) at six weeks of age. Further, reduced number of proliferating beta and alpha cells, decreased proportion of MafA+-beta cells and reduced insulin content was observed in islets of these mice, indicating that neonatal infections hindered appropriate beta cell maturation and thereby rendered mice longstanding glucose intolerant. In addition, the numbers of pancreatic macrophages upon S. aureus infection were reduced in comparison to that of control mice. Interestingly, transient macrophage depletion in the neonate resulted in impaired long-term islet function.

Conclusions: Taken together, our data show that bacterial infection during the essential phase of islet maturation in new-born mice decreased the pancreatic macrophage population and thereby, impaired islet development with long-term implications on glucose homeostasis.

P033-T | Microglial development and phagocytosis of apoptotic cells is mediated by PROS1

T. Burstyn-cohen; K. Zelentsova; A. Hochberg; S. Shtiglitz; A. Sznajderman

Hebrew University of Jerusalem, Jerusalem, Israel

Background: Microglia are the immune cells of the brain. As part of their function as immune regulators, microglia perform the phagocytic uptake of apoptotic cells, including the removal of apoptotic newborn neurons throughout life. Microglia also perform the ongoing task of synapse pruning, constantly contributing to neuronal plasticity. We study the role of PROS1 as an agonist for the tyrosine kinase receptor MERTK, which mediates phagocytosis of apoptotic cells.

Materials and methods: We selectively inhibited PROS1 expression from microglia by crossing LysM-Cre mice to homozygous to the floxed Pros1 allele. We assessed microglial function and development following Pros1 conditional knockout. The number of microglia and their ramifications were assessed. Microglial in-vivo phagocytosis of apoptotic cells was scored by the presence of phagocytic cups that have engulfed cleaved-caspase 3 immuno-positive cells. Microglial ramifications were quantified using Scholl analysis.

Results: Conditional knockout of PROS1 in microglia affects their development and number in adult brains, in a region-specific manner. While embryonic migration and colonization are normal in the absence of PROS1, the numbers of microglia and their complexity of processes is affected following PROS1 ablation. We find this is due to decreased proliferation and survival. Moreover, microglia lacking PROS1 are significantly less active in phagocytosis of cleaved-caspase 3-positive apoptotic cells.

Conclusions: PROS1 expressed by microglia is a novel regulator of microglia development and survival. Microglial PROS1 is also an agonist of phagocytosis of apoptotic cells, and thus is a key contributor to brain homeostasis.

P034-T | Preimplantation factor: a new regulator of neutrophil recruitment in vivo

R. Immler*; J. Tilgner†; M. Pruenster*; V. Morikis‡; A. Yevtushenko*; J. Grommes†; A.R.M. Kurz*; O. Soehnlein†,§; S.I. Simon‡; E. Barnea¶; M. Sperandio*

**Walter-Brendel-Center of Experimental Medicine, Biomedical Center, Ludwig-Maximilians Universität München, Munich, Germany; †Department of Vascular Surgery, University Hospital, RWTH Aachen, Aachen, Germany; ‡Department of Biomedical Engineering, University of California, Davis, USA; §Institute for Cardiovascular Prevention, Ludwig-*

Maximilians Universität München, Munich, Germany; ¶BioIncept LLC, Cherry Hill, New Jersey, USA

During pregnancy, the fetus has to be protected from being recognized and antagonized by maternal immune cells. At the same time, the maternal immune system has to operate normally to maintain regular functions. Preimplantation factor (PIF) is a small peptide that is only secreted by viable embryos already at early developmental stages, but can also be detected in maternal circulation throughout pregnancy. It is supposed to be involved in the regulation of maternal immune tolerance towards the embryo and therefore predicted to have immune-modulatory properties. PIF has been shown to have allaying effects in several autoimmune diseases. However, so far the mechanisms how PIF interacts with the immune system and which leukocyte subsets are targets of PIF are poorly understood.

To investigate the role of PIF on leukocyte recruitment in vivo, intravital microscopy of postcapillary venules in TNF-α stimulated cremaster muscle of wildtype mice was carried out. Here, we show that administration of PIF prior induction of inflammation significantly decreased leukocyte adhesion in inflamed cremaster and significantly reduced the number of extravasated neutrophils. A reduction in leukocyte adhesion could also be detected in in vitro flow chambers using murine whole blood preincubated with PIF. A reduction in the recruitment of leukocytes upon stimulation could be corroborated in an acute lung injury model after LPS inhalation. These findings can partially be explained by the observation that the presence of PIF impaired post-arrest modifications in vitro, namely neutrophil spreading and adhesion strengthening, accompanied by reduced levels of intracellular free calcium.

Taken together, our data show that PIF alters leukocyte recruitment by directly targeting neutrophils and by reducing their ability to adhere and/or extravasate into inflamed tissue. These findings identify PIF as an interesting therapeutic agent for disorders characterized by excessive recruitment of leukocytes as seen in several chronic and acute inflammatory diseases.

Supported by Deutsche Forschungsgemeinschaft SFB914.

P035-T | Uromodulin as a modulator of inflammation

M. Pruenster*; T. Steffen*; S. Bierschenk*; U. Keller†; R. Immler*; G. Hummel*; J. Scherberich‡; B. Uhl*; H. Mannell*; C. Reichel*; M. Sperandio*; B. Lange-Sperandio†

**Walter-Brendel-Center of Experimental Medicine, Biomedical Center, Ludwig-Maximilians Universität München, Munich, Germany; †von Haunersches Kinderspital, Department of Pediatric Nephrology Ludwig-Maximilians Universität München, Munich, Germany; ‡Klinikum Harlaching, Lehrkrankenhaus der Ludwig-Maximilians Universität München, Munich, Germany*

Uromodulin (UMOD), also known as Tamm–Horsfall glycoprotein (THP), is a renal glycoprotein produced by tubular epithelial cells in the thick ascending limb of Henle's loop. The peptide is primarily secreted apically into the tubular lumen, where it then fulfills its protective role against urinary tract infections and kidney stone formation. Interestingly, UMOD is also secreted basolaterally into the surrounding interstitial tissue, where it may exert immune-modulatory functions during renal inflammatory processes. Indeed, we found increased levels of UMOD in the obstructed kidney in a murine neonatal unilateral ureteral obstruction (UUO) model compared to the sham operated kidney. To further study the putative role of UMOD as a proinflammatory molecule we used intravital microscopy and visualized mouse cremaster muscle postcapillary venules of C57Bl/6 wild type (WT) mice after intrascrotal injection of UMOD or control buffer. UMOD-treated mice displayed reduced leukocyte rolling, reduced rolling velocities, and an increased number of adherent leukocytes in postcapillary venules of the mouse cremaster muscle. Additionally, UMOD increased the number of extravasated leukocytes and vascular permeability in vivo. In vitro experiments on HUVECs monolayers stimulated with UMOD revealed increased leakage of the endothelial monolayer and an increased number of transmigrating neutrophils across the monolayer underlining a proinflammatory role of UMOD. However, UMOD failed to directly activate β2 integrins on neutrophils and to induce upregulation of adhesion relevant molecules, like E-selectin, ICAM-1 and VCAM-1 on HUVECs suggesting that additional mechanisms contribute to the proinflammatory action of UMOD.

Supported by Deutsche Forschungsgemeinschaft SFB 914 and the FöFoLe-program of the Medical Faculty, LMU Munich.

MITOCHONDRIA, AGEING AND DISEASE

P036-T | Upregulated levels of clusterin in urine and kidney are peculiar for nephropathia epidemica

E. Garanina*; E. Martynova*; K. Ivanov*; A. Titova*; Y. Davidyuk*; V. Shakirova†; I. Khaertynova†; A. Rizvanov*; S. Khaiboullina*,‡

**Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical Academy, Kazan, Russian Federation; ‡University of Nevada, Reno, USA*

Background: Nephropathia epidemica (NE), milder form of hemorrhagic fever with renal syndrome (HFRS), is caused by Puumala hantavirus and characterized by the acute onset with high fever and malaise. The dominant clinical feature of NE is kidney insufficiency, which initially presents as the back pain and decreased urine output. Some patients develop anuria, which lead to the life-threatening complications. Histologically, hantavirus infection is defined as a tubule-interstitial nephritis with prominent leukocyte infiltration and interstitial hemorrhages. Despite the intensive research, clinically useful biomarkers of hantavirus caused kidney damage remain largely unidentified.

Materials and methods: Urine samples were collected from 64 NE patients hospitalized in the Republic Clinical Hospital for the Infectious Disease, Russia. All subjects provided at least one urine specimen, while 36 subjects provided a second urine sample. Additionally, urine samples were collected from patients diagnosed with other pathologies (systemic lupus erythematosus, gout characterized by renal dysfunction. The Institutional Review Board of the Kazan Federal University approved this study. Human kidney toxicity panels 1 and 2 (Bio-Rad, Hercules, CA) were used to analyze urine samples according to the manufacturer's recommendations. Histological analysis of postmortem collected kidney tissue from NE case was done using antibodies to hantaviral nucleocapsid and clusterin.

Results: Analysis of the early and late urine samples revealed upregulation of clusterin, KIM-1, IL-18 and CCL2 in NE as compared to control. Levels of calbindin, albumin, NGAL and osteopontin did not differ from that in controls. Upregulation of clusterin was detected in NE and SLE cases. Immunofluorescent analysis revealed extensive accumulation of hantavirus nucleocapsid protein and clusterin in kidney of fatal NE.

Conclusion: This data suggests that clusterin may play role in NE pathogenesis.

This work supported by Program of Competitive Growth of KFU.

Albert A. Rizvanov was supported by state assignment 20.5175.2017/6.7 of the Ministry of Education and Science of Russian Federation.

P037-T | Effects of multi-operational mitochondria-targeted antioxidants in human hepatic cells: promising drugs for liver disease

J.C. Teixeira*,†; R. Amorim*; R. Carreira*; F. Cagide†; S. Benfeito†; F. Borges†; P.J. Oliveira*

**CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Cantanhede, Portugal; †CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal*

Background: Mitochondrial redox balance is fundamental in controlling cellular life and death pathways. Antioxidants have been used to counteract redox networks disruption, associated with loss of cell homeostasis and disease pathophysiology, although therapeutic success is limited mainly due to pharmacokinetic drawbacks. Attempts to improve mitochondrial function spurred active drug discovery efforts and the development of new mitochondriotropic antioxidant agents, based on dietary polyphenols, has recently gained momentum. Phenolic acids are natural regulators of the cellular redox status and their antioxidant properties have pharmacological interest.

Material and methods: Human hepatocarcinoma cells were used to test the hypothesis that mitochondria-targeted agents, namely AntiOxCIN4 and AntiOxCIN6 present a multi-operational antioxidant mechanism of action. Thus, their beneficial effect in an in vitro model of liver disease was tested.

Results: AntiOxCINs toxicity was dose-dependent and only relevant for concentrations above those where antioxidant activity was observed. In fact, AntiOxCINs prevented oxidative stress-induced cytotoxicity without disturbing mitochondrial function, morphology and polarization, and intracellular ATP. Moreover, AntiOxCINs altered the redox state of the treated cells producing a mild increase in intracellular reactive oxygen species (ROS), which triggered an up-regulation of antioxidant defenses with no alterations on cell function and/or cell death. Instead, cellular GSH content was increased in cells treated with AntiOxCINs.

Conclusions: The development of mitochondria-targeted multi-functional antioxidants based on dietary scaffolds can stimulate stress responses and contribute to tissue protection, inhibiting directly or indirectly excessive mitochondrial ROS production is a promising strategy for drug development. It is likely that AntiOxCINs up-regulation of intracellular antioxidant defense system results in a beneficial effect by preventing mitochondrial excessive ROS generation associated with liver diseases.

Acknowledgements: This work was funded by FEDER funds through the Operational Programme Competitiveness Factors – COMPETE and national funds by FCT – Foundation for Science and Technology under research grants

PTDC/DTP-FTO/2433/2014, POCI-01-0145-FEDER-016659, POCI-01-0145-FEDER-007440.

P038-T | New amphiphilic polycationic derivatives of p-tert-butyl(thia)calix[4]arene – perspective DNA condensing agents for gene therapy

I. Antipin; V. Burilov; R. Ibragimova; G. Fatikhova; D. Mironova; E. Sultanova; S. Solovieva

Kazan Federal University, Kazan, Russian Federation

Gene therapy is an approach in which recombinant DNA delivery can result in ectopic expression of therapeutic gene and changes in gene expression landscape and protein levels in target cells and tissues. One of the important challenges in gene therapy is to search safe and efficient gene carries capable of compacting, protecting, transporting, and delivering nucleic acids into the cell. Most of condensing agents are functionalized on one side with appropriate cationic groups to act as DNA carriers, and, on the other side, with lipid-type chains that permit them to self-aggregate into well-defined aggregation patterns. Calixarenes and their thia-analogues are versatile compounds that can combine the properties of both macrocyclic hosts and self-organizing systems: micelles and liposomes. In addition, their relatively easy synthesis, variety of stereoisomeric forms and their low toxicity levels make them really promising vectors in gene delivery applications.

Herein, we demonstrate the synthesis and condensing abilities toward calf thymus DNA of new water-soluble amphiphilic derivatives of p-tert-butyl(thia)calix[4]arene in 1,3-alternate and cone stereoisomeric form with different O-alkyl lipophilic fragments (C4, C8, C14) containing two polycationic diethylenetriammonium fragments. Synthesized derivatives form stable vesicle-like aggregates in water solutions with the diameter within 50–100 nm and high zeta potential around +60 to +70 mV that corresponds to the formation of colloidal systems with high stability. According to the dynamical, electrophoretic light scattering data and fluorescent spectroscopy using ethidium bromide it was found that new polycationic macrocycles effectively interact with calf thymus DNA: a significant compression of DNA in 2–5 times was observed. Increased lipophilicity of the macrocycles leads to the formation of more compact lipoplex. Furthermore it was found that ethidium bromide removal can be promoted by hydrophobic interactions between the planar aromatic ethidium ring and the calixarene tail groups. Work was funded by RSF grant No. 14-13-01151.

P039-T | Amphiphilic carboxycalixresorcines as supramolecular nanocontainers for novel azo-dye-modified isatin derivative

I. Antipin*,†; Y. Morozova*,†; A. Bogdanov†; V. Syakaev†; A. Voloshina†; Y. Shalaeva*,†; A. Ermakova*,†; V. Zobov†; V. Mironov†

**Kazan Federal University, Kazan, Russian Federation; †A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan, Russian Federation*

The application of micelles of various amphiphilic compounds like polymers, lipids, surfactants for the formation of drug delivery systems and diagnostics solves the problem of the drug solubility, stability, biocompatibility, and increase of the retention time, but, as a rule, the loading efficiency of such nanocontainers is less than 10%. The amphiphilic supramolecular macrocycles such as calixarenes and calixresorcines can increase the drugs loading capability due to the numerous non-covalent interactions and formation of mixed nanoassociates with macrocycle/drug molar ratio of 1/1 and above.

Here we demonstrate the series of amphiphilic octacarboxycalix[4]resorcines (common formula I) which act as a supramolecular nanocontainers and effective solubilising agents for new poorly water soluble isatin derivative II. These macrocycles have zero or low hemolytic activities against human red blood cells (hRBC) and are able to increase the water solubility of the organic substrates by formation of mixed associates. Isatin derivatives belong to "so-called" privileged structures and are found applications in drug design and discovery. The preliminary experiments showed that new isatin derivative II bearing azo-dye moiety demonstrates the antibacterial and antifungal activities. The formation of the nanosized associates between the macrocycles and II is investigated by the UV-VIS, FT-PGSE NMR, 2D NOESY NMR, and DLS method. The efficiency of substrate loading in the supramolecular nanocontainers is of 30% achieved. Both this parameter and the size of assemblies formed depend on the macrocycle hydrophobicity. The driving forces of the guest loading and the structure of formed macrocycle-drug nanoassociates are discussed.

Work supported by Program of Competitive Growth of KFU and by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7).

P040-T | Generation and analysis of mesenchymal stem cell line stably expressing IL2

D. Chulpanova; V. Solovyeva; L. Tazetdinova; S. Kletukhina; A. Rizvanov
Kazan Federal University, Kazan, Russian Federation

Nowadays, cell and gene therapy are one of the most promising approaches for cancer treatment. Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells, which can be isolated from different types of adult tissues. Due to their tropism to the tumor niche, MSCs are promising vectors for the delivery of various antitumor agents. One of such agents is IL2 – immunomodulating cytokine, which regulates the activities of white blood cells.

In this study, human mesenchymal stem cells were isolated from adipose tissue. MSCs were transduced with recombinant lentiviral vectors encoding IL2 gene and Blue Fluorescent Protein (BFP) gene. Resulting cell lines were selected with blasticidin S (5 μg/mL) for 10 days. IL2 gene overexpression was confirmed by quantitative PCR. The viability of non-modified control and genetically modified with IL2 (MSC-IL2) and BFP MSCs (MSC-BFP) had no significant difference after 24, 48 and 72 hours. The percentage of apoptotic cells was determined by FITC Annexin V Apoptosis Detection Kit and PI (Sony, USA). There were 86% viable cells in wild-type control MSC culture, 89·9% in MSC-BFP and 91·4% in MSC-IL2 cultures after 24 hours. At 48 hours we observed 83% of viable cells in control MSC culture, 83·6% in MSC-BFP and 91·6% in MSC-IL2 cultures. All 3 MSC cultures were largely positive for mesenchymal stem cell surface markers including CD44, CD90, CD29, CD105, CD166 and CD73 and negative for hematopoietic stem cell surface markers CD34, CD11b, CD19, CD45, HLA-DR. The multipotency was confirmed via differentiation into chondrocytes, osteoblasts and adipocytes.

The antitumor activity of MSC-IL2 cells will be further investigated in various cancer cell cultures in vitro. The investigation was funded by RFBR grant 18-04-01133 and supported by Program of Competitive Growth of KFU.

P041-T | Viability and ultrastructure of human mesenchymal stem cells primed with cisplatin

L. Tazetdinova; Z. Gilazieva; S. Arkhipova; V. Solovyeva; E. Alekseeva; A. Gallyamov; D. Chulpanova; A. Rizvanov
Kazan Federal University, Kazan, Russian Federation

One of important tasks of translational medicine is increasing effectiveness of chemotherapy. Mesenchymal stem cells (MSCs) are a promising vector for the delivery of therapeutic agents, in particular chemotherapeutic drugs, to tumor niches. The tropism of MSCs to damaged tissues and tumor sites makes them suitable for therapeutic agent delivery to tumors and metastatic niches.

In this study MSCs were loaded with cisplatin for 24 hours at the concentrations: 10 μg/mL, 5 μg/mL or 2·5 μg/mL. The cytotoxicity of the drug was determined using MTS assay. The ultrastructure of MSCs after loading with the drug was analyzed using transmission electron microscope Hitachi HT7700 (Japan). It was shown that after 48 hours of incubation of MSCs with cisplatin, the toxic concentration was 10 μg/mL: the viability of the cells was 51·73% relative to the control untreated cells. Concentrations of 5 and 2·5 μg/mL had no toxic effect on MSCs: cell viability was 97·28% and 97·83%, respectively. Incubation of MSCs with cisplatin at concentrations 5 and 2·5 μg/mL did not lead to significant changes in the ultrastructure of the cells. After the incubation of MSCs with cisplatin at concentration 10 μg/mL, the number of pseudopodia cells significantly increased and the cisterns of the endoplasmic reticulum became enlarged, irregular or rounded, with more osmiophilic contents, which probably indicates a slight intoxication and a possible decrease in the synthetic function. At the same time, the number of autophagic vacuoles in the cytoplasm increased, multilamellar structures were defined within the vacuoles. Cisplatin did not affect the other ultrastructural features of MSCs, which indicates their relative resistance to a given concentration of cytostatic. The work was supported by grant from the Russian Foundation for Basic Research 16-34-60201 and Program of Competitive Growth of KFU.

P042-T | Histone H1.3 decrease efficiency of hantavirus infection in A549 cells in vitro

D. Chulpanova*; V. Solovyeva*; S. Khaiboullina*,†; A. Rizvanov*
**Kazan Federal University, Kazan, Russian Federation; †University of Nevada, Reno, Reno, USA*

In eukaryotic cells genomic DNA is densely packed around histones to form chromatin. The building blocks of chromatin are nucleosomal particles in which DNA are wrapped around octamers of core histones. Linker histone H1 binds to an octamers and consolidates the chromatin structure.

Hantaviruses are negative sense RNA virus belong to the family Bunyaviridae. These viruses cause two of diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). There is no specific

therapy for HFRS and HPS, so treatment remains symptomatic.

To determine the histone effect on hantavirus replication, A549 cells were infected with Prospect Hill virus (HPV) at MOI 0·1 in the presence of recombinant histone H1.3. Total RNA was collected and used to analyze virus replication using qPCR method. Expression of PHV S segment RNA was significantly decreased in A549 cells incubated with histone as compared to untreated mock infected cells. To determine the mechanism of inhibition of viral infection, we analyzed expression of the interferon regulated gene MxA, chemokines RANTES, IP10 and the apoptosis regulator Bcl2. H1.3 treatment did not affect expression of the MxA and Bcl2 genes in PHV infected as compared to untreated cells. However, expression of RANTES and IP10 was decreased in infected cells treated with histone H1.3.

Our data show that recombinant histone H1.3 suppresses PHV replication and decreases RANTES and IP10 expression in infected cell. RAA was supported by state assignment 20.5175.2017/6.7 of the Ministry of Education and Science of Russian Federation. Work supported by Program of Competitive Growth of KFU.

P043-T | The trophic role of mesenchymal stem cells in co-culture with neuroblastoma cells

K. Kitaeva; T. Prudnikov; A. Rizvanov; V. Solovyeva

Kazan Federal University, Kazan, Russian Federation

Stem cells are key participants in the regeneration processes of tissues and play an important role in the maintenance and formation of the tumor: mesenchymal stem cells (MSCs) have pronounced tropism to the tumor, mediating its growth, vascularization and development of chemoresistance. Thus investigation of the effect of stromal cells on the tumor can open new approaches for antitumor drugs screening. We investigated the trophic role of mesenchymal stem cells derived from human bone marrow (BM-MSCs) in co-culture with human neuroblastoma cells SH-SY5Y on extracellular matrix Matrigel and cell viability after incubation with cisplatin. Immunophenotyping of MSCs confirmed no markers of differentiated immune cells. BM-MSCs and SH-SY5Y cells were fluorescently labeled using Vybrant DiD (red) and DiO (green) dyes. Cell morphology and self-organization of cells in co-culture were analyzed using fluorescent microscope AxyObserver.Z1. After 120 hours co-cultured cells formed spheroids-like structures, consisting of clusters of cells. The FACS analysis of dissociated cultures showed high percentage of membrane component exchange between MSCs and tumor cells as determined by florescent spectra exchange. Cells viability/proliferative activity assay (MTS) was performed in a 96-well culture plate with a density of 5000 cells/well; to obtain a co-culture cells were mixed 1:1 with the same density. After 24 hours of incubation, the media was replaced and cisplatin added at 0·04 μg/mL to 10 μg/mL. After 24 hours of incubation with cisplatin MTS test was performed. Statistically significant ($P < 0·05$) increase in the viability of co-culture cells was noted after incubation with cisplatin at concentration of 10 μg/mL, in contrast to the monocultures of BM-MSCs and SH-SY5Y. Thus the study confirmed the significant role of MSCs in supporting of the growth and resistance of tumor cells. This study was funded by RFBR grant 16-34-60201. Work supported by Program of Competitive Growth of KFU.

P044-T | CCR5 genotype determines IL-9 and IL-32 secretion in nephropathia epidemica

E. Garanina*; K. Kletenkov*; E. Martynova*; Y. Davidyuk*; E. Kabwe*; E. Okhezin*; V. Shakirova†; I. Khaertynova†; A. Rizvanov*; S. Morzunov*,‡; S. Khaiboullina*,‡

**Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical Academy, Kazan, Russian Federation; ‡University of Nevada, Reno, USA*

Background: Nephropathia epidemica (NE), a mild form of hemorraghic fever with renal syndrome (HFRS), is caused by Puumala hantavirus. The lack of virus replication caused tissue damage in NE suggests that the NE pathogenesis is most likely the result of the organism reaction to viral infection. However, the molecular mechanisms of NE pathogenesis remain unclear. The mononuclear leukocyte infiltration in kidney tissue is commonly found in NE. Chemokine directed leukocyte migration is regulated by expression of the unique set of receptors, including CCR5 which is necessary for guiding the migration of activated and effector T cells.

Materials and methods: Chick swab samples from 592 donors, representing general population of the Republic of Tatarstan, and 98 NE cases were used for analysis of CCR5 genotype using PCR. Multiplex analysis of serum cytokines was performed using Bio-Plex Pro™ Human Cytokine 27-plex Assay and Bio-Plex Pro™ Human Inflammation Panel 1, 37-Plex (Bio-Rad, CA).

Results: Total of 492 (83·1%) donors of Tatarstan were determined as having wtCCR5 homozygous genotype, while 94 (15·9%) were heterozygous and only 6 (1·0%) were Δ32CCR5 homozygous.

In NE cases, the CCR5 genotype distribution was similar to that in general population, where 80 patients (81·7%)

had wtCCR5 homozygous genotype, 16 cases (16·3%) had heterozygous and 2 (2·0%) were Δ32CCR5 homozygous. Significant differences were found in serum levels of IL-9 and IL-32 between wtCCR5 homozygous and Δ32 heterozygous NE cases, where levels of IL-9 were higher (P = 0·011) and IL-32 were lower (P = 0·014) in homozygous as compared to Δ32 heterozygous NE.

Conclusions: This data suggest that CCR5 genotype determines intensity of immune response in NE.

This work supported by Program of Competitive Growth of KFU. Albert A. Rizvanov was supported by state assignment 20.5175.2017/6.7 of the Ministry of Education and Science of Russian Federation.

P045-T | Optimization of experimental and computational processes to study mitochondrial trafficking and metabolic biomarkers

R.F.V.L. Simões*; T. Cunha-Oliveira*; P.J. Oliveira*; F.B. Pereira[†,‡]

**CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Cantanhede, Portugal; [†]CISUC-Centre for Informatics and Systems, University of Coimbra, Coimbra, Portugal; [‡]Polytechnic Institute of Coimbra, Coimbra, Portugal*

Background: The complex polarity of neurons requires specialized mechanisms to allocate sufficient number of mitochondria to neurites and synapses to control local calcium and ATP levels. Deficits in trafficking are directly linked to neurodegeneration. Our objective is to demonstrate that alterations of mitochondrial spatial location and metabolism are interconnected and can lead to point-of-no-return situations characterized as the first signs of metabolic rupture.

Material and methods: The neuroblastoma cell line SH-SY5Y was used and differentiated using different protocols to develop a neuronal phenotype. To disturb mitochondrial metabolism and traffic, two mitochondrial poisons, rotenone and 6-hydroxydopamine, were used. Cells were labelled with different fluorescent probes and imaged under a INCell Analyzer. Cell mass and metabolic activity were measured using the SRB and resazurin assays, respectively.

Results: We have shown that a seven-day cell differentiation protocol using retinoic acid provided cells with better neuronal-like morphology, which are suited to study mitochondrial metabolic and dynamic parameters. We also demonstrate that rotenone and 6-hydroxydopamine toxicity is time and dose dependent, altering cell and mitochondria morphology, as well as mitochondrial membrane potential.

Conclusions: Images obtained with increasing concentrations of mitochondrial toxicants showed a gradual effect on different mitochondrial parameters that can be quantified using computational tools. These effects will be paired with metabolic data obtained for the same drug concentrations at later timepoints, to assess the consequences of mitochondrial disruption on cell viability. Building on the collected experimental information, we are training machine learning classification algorithms to accurately predict point-of-no-return situations that unbalance the cell to a state of metabolic catastrophe.

Funded by Montepio Foundation and FEDER/COMPETE/national funds by FCT under research grants PTDC/DTP-FTO/2433/2014, POCI-01-0145-FEDER-016659, POCI-01-0145-FEDER-007440. R.S. (PD/BD/128254/2016), T.C-O (SFRH/BPD/101169/2014) are supported by the European Regional Development Fund(ERDF) through COMPETE 2020 and Portuguese national funds via FCT–Fundação para a Ciência e a Tecnologia.

P046-T | Targeting dietary antioxidants to mitochondria ameliorate metabolic alterations of skin fibroblasts from patients with sporadic Parkinson's Disease

C.M. Deus*[,†]; S.P. Pereira*; T. Cunha-Oliveira*; S. Benfeito[‡]; F. Cagide[‡]; F. Borges[‡]; N. Raimundo[§]; P.J. Oliveira*[,†]

**Center for Neuroscience and Cell Biology, University of Coimbra, Cantanhede, Portugal; [†]IIIUC, Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; [‡]CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal; [§]Institute of Cellular Biochemistry, University Medical Center Goettingen, Goettingen, Germany*

Introduction: Parkinson's Disease (PD) is the most common movement disorder, characterized by dopaminergic neurodegeneration in the substantia nigra pars compacta. Although the cause of PD is still unclear, several studies showed that mitochondrial dysfunction plays key roles in dopaminergic neuronal loss. Consequently, mitochondrial function is an appealing target for pharmacological interventions. Mitochondrial dietary antioxidants have been studied as effective agents to control mitochondrial oxidative damage and improve mitochondrial function. We hypothesize that human skin fibroblasts represent a minimal-invasive method to detect metabolic and mitochondrial alterations in PD context, and to evaluate the pharmacological potential of novel phenolic acid-based mitochondria-targeted antioxidants to improve the PD phenotype in skin fibroblasts.

Materials and methods: A metabolic characterization of human skin fibroblasts from sporadic PD (sPD) patients and respective matched controls was performed by

measuring proliferation rates, metabolic viability, ATP levels, mitochondrial polarization, mitochondrial bioenergetics, oxidative stress, and SOD2 activity. Effects of new mitochondria-targeted antioxidants were determined using the same methods.

Results: Our results demonstrated that sPD fibroblasts have reduced metabolic viability with declined mitochondrial function characterized by a decreased oxygen consumption rate, lower mitochondrial polarization and ATP levels concomitant with increased mitochondrial oxidative stress and SOD2 activity, when compared to matched controls. Treatment of sPD fibroblasts with non-toxic concentrations of dietary antioxidants improved mitochondrial function and made sPD fibroblasts physiologically more similar to the matched controls.

Conclusions: Our data show that the impaired mitochondrial bioenergetics and the defective metabolism that typically characterize the neurodegenerative process in PD can be detected in sPD fibroblasts. We also demonstrate that novel mitochondria-directed antioxidants increase metabolic viability of sPD cells. Human fibroblasts may represent a minimally invasive tool to study altered metabolism and new treatment strategies in PD.

Acknowledgements: Funded by Montepio Foundation, FCT-Portugal grants (PTDC/DTP-FTO/2433/2014, CMD: SFRH/BD/100341/2014, TCO:SFRH/BPD/101169/2014 and SPP:SFRH/BPD/116061/2016), co-funded by FEDER/COMPETE/National Funds (POCI-01-0145-FEDER-016659, POCI-01-0145-FEDER-007440).

P047-T | Changes of the pancreas in experimental diabetes mellitus in rats after administration of alloxan

A. Titova*; M. Mavlikeev*; M. Kaligin*; D. Syleimanova*; A. Kiyasov*; R. Deev[†,‡]

**Kazan Federal University, Kazan, Russian Federation; [†]Institute of Human Stem Cells, Moscow, Russian Federation; [‡]Ryazan State Medical University named after academician I.P. Pavlov, Ryazan, Russian Federation*

Most studies propose the model of chemically induced diabetes mellitus, which is reproduced by the administration of alloxan despite of controversial data on pancreas damage. The purpose of our investigation was to describe the early micro and ultrastructural changes that occur in the pancreas due to the administration of alloxan.

Alloxan was administered to rats intraperitoneally at a dosage of 180 mg/kg. The material for electron microscopy and pathohistological analysis was taken at 12, 24 and 48 hours post-injection.

Pathohistological analysis of the pancreas following changes in the acini increased, the exocrinocytes overgrown by the zymogen. The ducts were overcrowded with a thick secret, overgrown, in some channels an atrophic epithelium was observed. The absence of boundaries between endocrine cells, dystrophy and degranulation of islet cells, karyotypic cannitis and karyolysis of islet cells were shown.

Ultrastructural changes observed in some parts of the gland characterizing massive damage of mitochondria. In exocrine cells, mixed large-droplet fatty dystrophy was observed. In the central part endocrine cells exhibited absence of nuclei or their remains. The boundaries of endocrinocytes were partially destroyed, which resulted in the fusion of cells with the formation of multinuclear structures.

The area of islets increased due to the damage development. Immunofluorescent staining revealed increased number of insulin+ and caspase-3+ cells at 12 and 24 hours, though at 48 hours the number of caspase-3+ cells gradually decreased.

The described ultra- and microstructural changes testify to the significant contribution of apoptosis to endocrinocyte death in alloxan damage, as opposed to previous studies, which took into account only necrotic changes. In addition, we primarily described concomitant lesions of the exocrine part of the pancreas with involvement of its stroma.

Work supported by Program of Competitive Growth of KFU.

P048-T | Mitochondriotropic cinnamic acid antioxidants increase cellular stress responses in HepG2 cells: involvement of mitochondrial ROS signaling in a possible therapy for hepatic steatosis?

R. Amorim*[,‡]; J. Teixeira*[,‡]; S. Benfeito*; F. Cagide*; J. Garrido*[,†]; P. Oliveira[‡]; F. Borges*

**CIQ/ Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Porto, Portugal; [†]Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto, Porto, Portugal; [‡]CNC – Center for Neuroscience and Cell Biology, Coimbra, Portugal*

Background: Non-alcoholic steatohepatitis (NASH), the deleterious form of non-alcoholic fatty liver disease, is a main health problem in industrialized countries. Mitochondrial dysfunction plays a key role in the pathology of NASH. Increased mitochondrial generation of reactive oxygen species (ROS) was observed in NASH animal models, which makes this event an attractive target for pharmacological and non-pharmacological intervention. We

developed novel mitochondrial-directed antioxidant based on naturally occurring phenolic acids. Our present objective is to investigate whether these novel molecules prevent mitochondrial and cellular damage on an in vitro lipotoxicity model.

Material and methods: We studied the effects of the novel mitochondriotropic agents (AntiOxCIN4 and AntiOxCIN6) on human hepatoma-derived cell line HepG2 incubated with supra-physiological concentrations of palmitic acid (PA), measuring cell mass and cytotoxicity, oxidative stress, caspase 3- and 9-like activities, glutathione (GSH) content, peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1-α) and superoxide dismutase (SOD) protein levels, and lipid content.

Results: Our data showed that positively charged cations with catechol moieties presented higher toxicity than those containing a pyrogallol moiety in their structure. After 48 hours of treatment, AntiOxCIN4 caused an increase of HepG2 cell mass and induced a transient increase in intracellular ROS without triggering pro-apoptotic responses. AntiOxCIN4 also prompted a ROS-dependent stimulation of the endogenous antioxidant defense system and mitochondrial biogenesis, as measured by an increase in GSH content and PGC1-α expression, respectively. AntiOxCIN4 also partly prevented lipid accumulation induced by PA.

Conclusions: Mitochondriotropic antioxidants based on dietary scaffolds and with complementary antioxidant mechanisms can be used as therapeutic agents in the treatment of oxidative stress-related conditions, including liver diseases

Funded by FEDER funds through COMPETE and national funds by FCT – Foundation for Science and Technology under research grants PTDC/DTP-FTO/2433/2014, POCI-01-0145-FEDER-016659, POCI-01-0145-FEDER-007440. J.Teixeira (NORTE-01-0145-FEDER-000028), R.Amorim (SFRH/BD/131070/2017) grants are supported by the European Regional Development Fund (ERDF) through COMPETE 2020 via FCT.

P049-T | Histochemical analysis of self-organizing 3D spheroid-like cultures of adipose-derived mesenchymal stem cells

A. Shafigullina; A. Titova; A. Rizvanov; A. Gumerova; A. Kiassov

Institute of Fundamental Medicine and Biology, Kazan (volga Region) Federal University, Kazan, Russian Federation

Growth of the cells within cell culture in vitro is regulated by contact inhibition. When the cells form monolayer and contact with neighboring cells, they stop proliferation, migration and remain quiescent. According to published data adipose-derived mesenchymal stem cells (AD-MSC) do not follow this rule, grow above each other, form focuses. Interestingly, in our culture late passage cells organized in 3D spheroid-like structures in vitro. The aim of the project was to study cell within these spheroids.

AD-MSC were isolated from rat visceral adipose tissue by collagenase digestion, cultivated in standard conditions. AD-MCS monolayer culture was Ki-67+, α-SMA+, desmin-low, CK19-. Some spheroids, formed by AD-MSC of the passage 12 were fixed in formalin. Paraffin slices were stained histochemically with hematoxylin-eosin and immunohistochemically with antibodies against Ki-67 (proliferation), α SMA (migration), desmin, CK19 (epithelial cell marker). Remaining spheroids were placed in a 6-well plate to check viability and possibility of cells for explantational outgrowth.

Histochemical staining demonstrated fusion of cells with formation of syncytium-like structures. On the periphery cells were polygonal, round, had epithelial morphology. Groups of cells organized in line inside spheroid remained elongated AD-MSC shape. Most of the cells did not proliferate. There were no migrating α SMA+ cells. Polygonal cells on the periphery and a few cells inside the spheroid were desmin+. Interestingly, peripheral and elongated shape cells expressed epithelial marker CK-19+. Probably, within spheroid there was spontaneous mesenchymal-epithelial transdifferentiation. When spheroids were placed in a new culture dish we observed explantational outgrowth of spindle-shaped AD-MSC suggesting that cells within spheroids were alive, able to outgrow and revert through epithelial-mesenchymal transdifferentiation back to MSC phenotype.

Investigation was funded by RFBR grant 18-04-01133 and supported by Program of Competitive Growth of KFU.

P050-T | Isocitrate dehydrogenase 2 deficiency exacerbates dermis damage by ultraviolet-B

H.J. Ku; J.H. Park; J.-W. Park

Kyungpook National University, Taegu, South Korea

Isocitrate dehydrogenase 2 (IDH2) is a key enzyme that maintains the balance of mitochondrial redox status by generating NADPH as a reducing factor, which is used to reduce oxidized antioxidant proteins and oxidized glutathione. Therefore, the role of IDH2 is crucial in organs that are easily influenced by reactive oxygen species (ROS) or mechanical damage. Humans are constantly exposed to ultraviolet (UV) radiation throughout their lifetime, which

can cause various cutaneous diseases, such skin carcinoma, dermatitis, and sunburn. ROS play an important role in the initial step of these diseases; therefore, IDH2 deficient mice (KO) could be a useful model to investigate UV-mediated skin damage. When we exposed the dorsal skin of KO mice to UVB, pyrimidine dimers and (6-4) photoproducts (6-4PPs), marker of photoproducts generated by UVB, were found in the dermis of the knockout mice. Increased collagen degradation, apoptosis, inflammation, and ROS levels in the dermis were also observed. These results indicated that UVB could reach the dermis by penetrating the epidermis. We then attempted to determine how the epidermis was breached, and observed a decrease in the expression level of deltaNp63, a major protein required for epidermis generation, in the KO mice. The mito-TEMPO supplement significantly ameliorates UVB-induced damage in the skin of KO mice. In the present study, we provided a role for IDH2 in protection against UVB-induced skin damage and a new connection between IDH2 and deltaNp63.

P051-T | Effects of the caffeine on the snails training through the regulation of Ca^{2+} concentration trough ryanodine receptors of the endoplasmic reticulum and mitochondria

T. Bogodvid*,†; D. Silantieva*; V. Andrianov*; L. Muranova*; I. Deryabina*; K. Gainutdinov*

**Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russian Federation; †Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russian Federation*

Calcium ions play a critical role in the regulating of a great variety of neuronal processes due to their specific physicochemical characteristics. They are the most versatile intracellular mediator linking the processes, developing in the surface membrane of the cell, and reactions occurring within the cell. It is generally accepted that intracellular calcium, which is ionized, has the function of universal secondary mediator involved in the regulation of many intracellular reactions, down to gene expression. One of the agents that cause the increasing of intracellular concentration of Ca^{2+} is caffeine, which acts on the ryanodine receptors in the endoplasmic reticulum and mitochondria. That is, the regulation of intracellular concentration of Ca^{2+} ions occurs through these receptors. In this regard, the aim of our study was investigate the effects of chronic administration of caffeine on the formation of conditioned defensive reflex in snails.

It was used 5 groups of animals: intact (n = 18), snails, received a saline injection 30 minutes before training (active control, n = 11), snails, injected with caffeine 30 minutes before training (n = 18), snails injected with caffeine immediately after the training (n = 18), snails, which were injected daily with caffeine during the study period (n = 8). Our experiments showed that the chronic injection of caffeine increases elaboration of defensive conditioned reflex in snail. The injection of caffeine immediately after the training procedure was produced learning faster than when caffeine applied before training. Thus, changes in intracellular Ca^{2+} ions concentration by caffeine, which occurs by activation of the ryanodine receptors of the endoplasmic reticulum and mitochondria serves as regulator of plastic processes during learning.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University (No. 17.9783.2017/8.9) and supported by RFBR (grant No. 18-015-00274).

P052-T | Dynamics of nitric oxide production in the rat hippocampus as one of the mechanisms of inflammation during ischemic and hemorrhagic insult

T. Bogodvid*; V. Andrianov*,†; G. Yafarova*,†; V. Iyudin†; S. Pashkevich‡; M. Dosina‡; T. Baltina*; V. Kulchitsky‡; K. Gainutdinov*,†

**Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russian Federation; †Zavoisky Physical-Technical Institute of RAS, Kazan, Russian Federation; ‡Institute of Physiology of NAS Belarus, Minsk, Belarus*

It is known that nitric oxide (NO) and peroxynitrite are involvement in the pathophysiology of well-characterized acute and chronic inflammatory diseases and play an important role in the development of organ damage and inflammation triggered by various drugs and chemical agents (Patcher et al., 2007). Those processes characterized the hypoxia that occurs including the development of tissue ischemia. According to this the study of the pathogenesis, the methods of correction and the mechanisms of stroke is important both from the theoretical and practical points of view. The main purpose of our investigation was to study the dynamics of NO production in the hippocampus of rats after modeling both ischemic and haemorrhagic stroke.

Electron paramagnetic resonance (EPR) was used as a method to record NO production in the tissues of the brain of healthy rats and rats after modeling of ischemic and haemorrhagic stroke. Direct measurement of the dynamics of NO production by EPR spectroscopy in our experiments showed that after the emergence of signs of ischemic stroke, 5 hours after the start of ischemia, the content of

NO in the hippocampus decreased 2–3 times and this decrease is maintained at 24 and 72 hours.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University (No 17.9783.2017/8.9) and supported by RFBR (grant No. 16-04-00098).

P053-T | Effects of inhibition of tryptophan hydroxylase synthesis on context memory

T. Bogodvid*,†; I. Deryabina*; V. Andrianov*; L. Muranova*; D. Silantieva*; K. Gainutdinov*

**Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russian Federation; †Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russian Federation*

It is established that serotonin (5-HT) is a basic neurotransmitter for defensive behavior in mollusks and learning on the basis of defensive reflexes. In behavioral experiments it was shown that the disruption of serotoninergic system by the neurotoxin 5·7-DHT did not change the original memory, however, led to a memory impairment after repeated reactivation. An unavailability of reactivation under the action of the antagonist of serotonin receptors methiothepin was also shown. These results demonstrated the relevance of analysis of long-term memory after inhibition of 5-HT synthesis. Tryptophan hydroxylase (TPH) is the first and presumably rate-limiting enzyme in 5-HT biosynthesis. P-chlorophenylalanine (p-CPA) is one of the various drugs that depresses TPH, it causes long and deep depletion of brain 5-HT-depots.

Here, we investigated the possible changes of the reconsolidation under the conditions of 5-HT deficit, caused by injection of inhibitor of TPH synthesis (intermediate stage of the synthesis of 5-HT) p-CPA. It was shown that the forgetting process for conditioned situational reflex after reminder and inhibition of protein synthesis did not occur if the 5-HT transmission in nervous system was impaired. This effect was significantly different from the direct action of inhibitor of protein syntheses anisomycin, which completely blocked the reconsolidation of context memory. We concluded that the 5-HT system was included to the process of memory reconsolidation (in our system of situational memory).

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University (No. 17.9783.2017/8.9) and supported by RFBR (grant No. 18-015-00274).

P056-T | Cytotoxicity of doxorubicin-loaded PLGA and PLGA-PEI nanoparticles: relevance for anti-tumor effects and cardiotoxicity

C. Candido*; L. Ferreira†; E. Silva‡; A. Júnior‡; R. Peccinini*; P. Oliveira†

**Department of Natural Active Principles and Toxicology, São Paulo State University (Unesp), School of Pharmaceutical Sciences, Araraquara, Brazil; †CNC, Center for Neuroscience and Cell Biology, University of Coimbra, UC-Biotech-Biocant Park, Cantanhede, Portugal; ‡Laboratory of Pharmaceutical Technology and Biotechnology, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Rio Grande do Norte, Natal, Brazil*

Background: Doxorubicin (Dox) is an antineoplastic antibiotic widely used in therapeutics, although its high cardiotoxicity limits its use. Our objective was to use pharmaceutical technology to improve Dox biodistribution and consequently reduce toxicity, without sacrificing the antitumoral efficacy. We developed two Dox delivery nanoparticle systems of poly (D,L-lactide-co-glycolide)-based nanoparticles (PLGA-NPs) and PLGA-NPS bearing surface polyethyleneimine (PEI/PLGA-NPs) and tested cytotoxicity on human breast tumor cells (MDA-MB-231 and MCF-7), normal human breast cells (MCF-12A) and rat cardiomyoblasts (H9c2).

Material and methods: Tumor and normal cell lines were incubated with free Dox, Dox-PLGA-NPs and Dox-PEI/PLGA-NPs for 24 hours (0·1–2·0 μM Dox). Cytotoxicity analysis was performed by resazurin, sulforhodamine B assay, and determination of ATP levels. p53 levels were detected by immunoblotting.

Results: Cytotoxicity assays demonstrated that all Dox formulations induced dose-dependent cell death in the tested cell lines (with exceptions for MDA-MB-231). No differences in cell death were observed between free Dox and the NP systems in the normal cell lines. However, for the MCF-7 cell line the Dox-PEI/PLGA-NPs showed higher reduction of cell mass vs observed in free Dox or Dox-PLGA-NPs treatments. Although no statistically differences were observed between the different treatments on H9c2 cells, with the NPs systems p53 levels were decreased by ap. 30% when compared with free Dox.

Conclusion: Dox chemotherapy efficiency is compromised by the cardiac side effects and drug delivery systems have been explored in an attempt to overcome off-target toxicity. We tested the cytotoxicity of two NPs systems for drug encapsulation and observed increased toxicity of Dox-PEI/PLGA-NPs in the breast tumor cell line MCF-7, while both NPs formulation seem to lower the activation of p53 in the cardiomyoblast cell line.

The study was supported by CAPES (ref.88881.132300/2016-01); FCT (PTDC/DTP-FTO/1180/2012, SFRH/BD/52

429/2013 to LF) and FEDER /COMPETE (POCI-01-0145-FEDER-007440, CENTRO- 07-ST24-FEDER-002008).

P057-T | Crosstalk between H_2S and glutathione in cardioprotection against ischemia/reperfusion in rats

Y. Goshovska; R. Fedichkina; V. Sagach

Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv, Ukraine

Glutathione is an essential molecule for antioxidant defense. It is synthesized by glutamate-cysteine ligase enzyme (GCL) from L-cysteine which alternatively might be used by cystathionine-gamma-lyase (CSE) for production of hydrogen sulfide (H_2S). H_2S donors demonstrate protective effect against myocardial ischemia/reperfusion (I/R) injury. However, the role of endogenous H_2S remains unclear. In this work we used CSE and GCL inhibitors (D, L-propargylglycine (PAG) and D,L-buthionine-(S,R)-sulfoximine (BSO) in order to modulate the metabolic pathways of L-cysteine in cardiac I/R.

Wistar rats were treated with PAG(11·3 mg/kg)+L-cysteine (121 mg/kg), BSO(22·2 mg/kg)+PAG+L-cysteine. Cardiodynamics and oxygen cost of myocardial work of Langendorff isolated heart were studied. Mitochondrial membrane permeability transition (MPT) was evaluated by UV spectra absorbance of effluent collected from the pulmonary artery. Heart tissue was examined for GSH and GSSG levels with Elman's reagent. Generation rate of hydroxyl radical, superoxide radical, H_2S and diene conjugates content were also measured.

Simultaneous application of PAG and L-cysteine evoked powerful cardioprotective effect in terms of 90–100% recovery of contractile activity and coronary flow, absence of drastic increase of end-diastolic pressure, etc. I/R-induced oxidative stress and non-effective oxygen utilization by myocardium as well as MPT pore opening were significantly lower in PAG+L-cysteine group. Our data showed that I/R induced decrease in cardiac GSH and GSSG levels by 49% and 44% respectively ($P < 0{\cdot}03$). Pretreatment with PAG+L-cysteine increased the basic levels of GSH and GSSG in 3- and 2-times respectively. Despite of I/R, GSH and GSSG levels were 3-times higher than in non-treated rats, thus, increasing antioxidant status of myocardium. PAG+L-cysteine prevented increase of H_2S content induced by I/R (200% vs 551% in non-treated, $P < 0{\cdot}001$). The cardioprotective effect of PAG+L-cysteine was completely abolished by an inhibitor of glutamate-cysteine ligase enzyme BSO.

Thus, we demonstrate the reverse relation between glutathione and H_2S that might be used for induction of cardioprotection.

P058-T | Interplay between tissue-specific mitochondrial function and whole body glucose homeostasis

P. Gama-Pérez*; A. Gonzalez-Franquesa†; S. Moreno*; P. Rullens*; P. Garcia-Roves*,‡

**Department of Physiological Science, University of Barcelona, Barcelona, Spain; †Section for Integrative Physiology, The Novo Nordisk Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark; ‡Institut d'Investigacions Biomèdiques de Bellvitge (IDIBELL), Barcelona, Spain*

There is a close connection between mitochondrial functionality and the development of metabolic pathologies such as obesity-related type 2 diabetes. However, the widespread hypothesis suggesting a causality link between mitochondrial dysfunction and insulin resistance has been debated repeatedly and is still controversial (i). Indeed, genetic models of obesity have proved a differentially adaptive mitochondrial response according to the metabolic demands in a tissue-specific manner (ii). Mimicking this pathophysiological situation, we used a model of diet-induced obesity (DIO) for the integrative assessment of metabolic plasticity, as well as for its potential reversibility through a lifestyle intervention. Thus, our main aim is to assess whether tissue-specific mitochondrial adaptations due to obesity-related type 2 diabetes are also present in a more physiological context.

We evaluated in DIO mice, before and after the lifestyle intervention, different parameters related to its pathological phenotype such as body weight, body composition and glucose homeostasis. We also analyzed, using high-resolution respirometry (Oroboros Instruments®) (3), mitochondrial OXPHOS capacity in the main insulin-sensitive tissues (liver, oxidative and glycolytic skeletal muscle and epididymal and subcutaneous adipose tissue).

Regarding mice phenotype, body composition and glucose homeostasis our data showed that the pathological state in our model strongly resemble obesity-related T2DM and our lifestyle intervention significantly reverted this pathological-associated alterations. Moreover, tissue-specific analysis of mitochondrial function showed clear differences in its behavior among groups, supporting the idea that 1) mitochondrial dysfunction is not a common shared feature in states of whole body insulin resistance, and 2) there may be tissue-specific mechanisms governing mitochondrial performance leading to the adaption of mitochondria in different manners.

1. Montgomery MK and Turner N. Endocr Connect 2015 4(1):1–15.
2. Hollmström MH, Iglesias-Gutierrez E, Zierath JR, Garcia-Roves PM. Am J Physiol Endocrinol Metab 2012 302(6): 731–739.
3. Cantó C and Garcia-Roves PM. Curr Protoc Mouse Biol 2015 5(2): 135–153.

P059-T | Structural and functional alterations of platelets induced by autoimmune anti-DNA antibodies

I. Andrianova*; E.E.C. Agboigba*; R. Litvinov*,†

*Kazan Federal University, Kazan, Russian Federation; †University of Pennsylvania School of Medicine, Philadelphia, USA

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease associated with a high risk of venous and arterial thrombosis, which may be due to continuous platelet activation in the blood. Because SLE is associated with high levels of anti-dsDNA autoantibodies (Abs) in blood, we hypothesized that these Abs or their immune complexes could directly activate platelets perhaps via Fc-receptors.

Material and methods: To test the hypothesis, we modeled pathogenic effects of purified anti-dsDNA Abs and immune complexes formed by DNA and anti-dsDNA Abs on isolated normal platelets. Scanning electron microscopy and flow cytometry were used to characterize the morphology and functionality of platelets in the absence or presence of anti-dsDNA Abs or their complexes with DNA.

Results: Flow cytometry showed that platelets incubated with isolated anti-DNA Abs or with the DNA/Abs complexes had a significantly higher level of *P*-selectin expression than control untreated platelets. In contrast, platelets pre-incubated with a monoclonal antibody against the FcγRIIA receptor and then treated with anti-dsDNA Abs or DNA-containing immune complexes had a low *P*-selectin expression level close to that of control. Electron microscopy of platelets incubated with anti-dsDNA Abs or the DNA/Abs complexes revealed morphological characteristics of activation: multiple filopodia, reduced platelet body volume and formation of small platelet aggregates. In the presence of an anti-FcγRIIA antibody the morphology of platelets remained unchanged by anti-DNA Abs alone or in a complex with DNA and was similar to control untreated platelets that had discoid shape with very few filopodia.

Conclusions: The results suggest that platelets can be activated through the FcγRIIA receptor by anti-dsDNA Abs alone or by the complexes containing DNA and anti-dsDNA Abs. The immune platelet activation can promote hypercoagulability and sustain the prothrombotic status in SLE patients. Work supported by the Program of Competitive Growth at Kazan Federal University.

P060-T | Analysis of proinflammatory cytokines in patients with multiple sclerosis

E. Garanina*; E. Martynova*; A. Rizvanov*; S. Khaiboullina*,†; T. Khaiboullin‡

*Kazan Federal University, Kazan, Russian Federation; †University of Nevada, Reno, USA; ‡Republican clinical neurological center, Kazan, Russian Federation

Background: There are 3 million multiple sclerosis (MS) cases are registered worldwide. The highest incidence of MS is found in Northern Europe, North America and in southern Australia. The mechanisms of MS pathogenesis are largely unknown. However, the role of the chronic inflammation and activated immune response is suggested in MS onset and progression.

Material and methods: Serum samples were collected from 20 patients diagnosed with MS at the Republican Clinical Neurological Center of the Republic of Tatarstan. In addition, serum samples were collected from 20 controls. All specimens were acquired as existing de-identified surplus clinical diagnostic specimens, under an exemption to institutional review board (IRB).

Serum and CSF cytokines were analyzed using Bio-Plex multiplex magnetic beads (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Bio-Plex Pro Human Cytokine 27-plex and Bio-Plex Human Cytokine 21-plex panels were used.

Results: Total of 48 cytokines were analyzed. Only levels of 9 cytokine differed between MS and control serum: IL-17, IL-12 (p40), CCL2, CCL3, CCL4, CCL5, CXCL10, MIF and TRAIL. These cytokines are associated with activation and chemotaxis of leukocytes. STRING data analysis suggests that upregulation of TRAIL activates CCL2, which then activates CCR5. CCR5 is positioned in the middle of the activated cytokines cluster and interacts with each analyte. These data suggest that CCR5 plays a central role in the pathogenesis of MS by supporting the inflammation and leukocyte migration.

Conclusions: Data on cytokine activation in MS indicates their role in MS pathogenesis and explain the mechanisms of inflammation and leukocyte activation.

This work supported by Program of Competitive Growth of KFU. Albert A. Rizvanov was supported by state assignment 20.5175.2017/6.7 of the Ministry of Education and Science of Russian Federation.

P061-T | Possibilities of application of photodynamic therapy for the treatment of squamous cell carcinoma of the esophagus

S. Zinchenko*; A. Ivanov†; A. Bilyalov*; R. Gaifullina*

Kazan Federal University, Kazan, Kazan; †Tatarstan Cancer Center, Kazan, Kazan

Background: Limitations of photodynamic therapy in providing high-tech medical care and the relatively expensive photosensitizer in most cases constitute an obstacle to the full implementation of the method in the clinical practice. Thus, the aim of our report is to describe the clinical case of effective use of the method of photodynamic therapy as a method of choice in a patient with synchronous locally advanced cancer of the esophagus and lung.

Materials and methods: Patient V., 68 years old, was observed in the «Tatarstan Cancer Center» (Kazan, Russia) from December 2011 with a diagnosis of cancer of the larynx T3N1M0 after successful complex treatment. In March 2017 patient was diagnosed squamous cell carcinoma of the esophagus and right lung. The decision was made to carry out treatment using a method of photodynamic therapy. Photodynamic therapy was carried out by the apparatus Latus 662 nm (ООО «Аткус», Russia) using of lightguide (diameter of 400 mm.) with output beam power of 1·4 kW with 8 minutes. exposure (summed energy 150–180j) after injection of 0·35% solution of radachlorin (ООО «Рада-Фарма, Russia») in the amount of 1 mg/kg of body weight. The procedure was performed without general anesthesia.

Results and discussion: Marked persistent (6 months) stabilization of synchronous neoplastic process in the background system of monotherapy with etoposide and two-time endoscopic photodynamic therapy of tumors of the esophagus and lung.

Conclusions: Thus, photodynamic therapy for locally advanced synchronous tumors of the esophagus and lung is the method of choice of palliative therapy to achieve a good survival rate at high quality of life.

Work supported by Program of Competitive Growth of KFU.

P062-T | Puncture treatment of acute pancreatitis

S. Zinchenko*; A. Zainytdinov*; I. Malkov†

Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical Academy, Kazan, Russian Federation

Background: The use of minimally invasive manipulation under control of ultrasound and computed tomography allowed to revise the outlook on the treatment of pancreatic necrosis

Materials and methods: In the treatment was 87 patients with infected pancreatic necrosis proven. Achieved 121 punctures and drainage of omental and retroperitoneal space.

Results and discussion: In 16 patients in the group of moderate severity were 4% of early toxemical complications in the form of acute circulatory failure, pneumonia, and 5% postnecrotic late complications such as abscesses paracolic space, festering wounds, intestinal fistulas. In 5 patients in group-severe toxemical early complications were 10%, mainly in the form of pneumonia, and later postnecrotic complications of retroperitoneal abscesses, festering wounds, intestinal fistulas, bleeding arrosive totaled 14%.

Conclusion: Thus, under ultrasound guidance puncture recommend spending when infected fluid clusters depending on the clinical situation and ultrasonography protocol. A promising direction in the prevention and treatment of bleeding in arrosive performing punctures and drainage of abdominal ultrasound formations control at destructive pancreatitis is the use of endovascular x-ray surgery.

Work supported by Program of Competitive Growth of KFU.

P063-T | Ultrastructural changes in platelets induced by immune complexes containing platelet factor 4

E. Mordakhanova*; A. Ponomareva*; T. Nevzorova*; R. Litvinov†

Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russian Federation; †Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, USA

Background: Heparin-induced thrombocytopenia (HIT) is a complication of heparin therapy. HIT is caused by pathogenic anti-platelet factor 4 (PF4)/heparin antibodies and characterized by a low platelet count associated with thrombosis. Cellular mechanisms of thrombosis in HIT are not fully understood, but platelet activation is thought to be the main pathogenic factor. Here, we studied ultrastructural changes in platelets treated in vitro with immune complexes containing pathogenic anti-human-PF4/heparin antibodies (named KKO) and platelet factor 4.

Material and methods: Gel-filtered isolated normal human platelets were incubated with recombinant human PF4 or monoclonal KKO antibodies or the KKO/PF4 complexes for 15 or 60 minutes followed by fixation and preparation for transmission electron microscopy. Platelets treated with calcium ionophore A23187 were used as a

positive control for activation and untreated platelets were used as a negative control.

Results: Transmission electron microscopy showed that platelets treated with KKO/PF4 or A23187, unlike untreated cells, displayed dramatic morphological changes. The KKO/PF4-treated platelets had irregular non-discoid shape, formed membrane invaginations and protrusions; they had many intracellular vacuoles and enlarged lumens of the open canalicular system. The vacuoles contained various inclusions, such as secretory granules, membrane components, and loose-grained enclosures. The number of secretory granules in the KKO/PF4-treated cells was dramatically reduced. In all cases, we observed formation of extracellular macrovesicles of various shape and size. The changes in KKO/PF4-treated platelets were much more pronounced after 60 minutes of incubation compared to 15 minutes. KKO or PF4 applied separately caused similar structural alterations in platelets as the KKO/PF4 complex, but fewer platelets were affected and the structural changes were much less pronounced.

Conclusions: The results show that PF4-containing pathogenic immune complexes induce a strong and time-dependent platelet activation, which can promote thrombosis in HIT. Work supported by the Program for Competitive Growth at KFU.

P064-T | Effect of betulin derivatives on platelet aggregation

I. Antipin*,†; O. Tsepaeva†; A. Nemtarev*,†; L. Grigoréva*; L. Ziganshina*; H. Cong*; T. Abdullin*; V. Mironov*,†

Kazan Federal University, Kazan, Russian Federation; †A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan, Russian Federation

Recent reports show that platelets play fundamental role in cancer progression. Cancer cells can promote activation and aggregation of platelets, which interact with the cells and release highly active molecules, such as growth factors, to enhance tumor growth, angiogenesis, metastasis and resistance to immune attack.

Triterpenoid Betulin and its derivatives (C-28-betulin-esters: C-28-bromoalkanoyl(C3,C4)- (compounds 1,2); C-3,C-28-betulin-diesters: C-3,C-28-di(bromoalkanoyl(C3,C4)- (compounds 3,4); C-28-betulin-triphenylphosphonium(TPP) conjugates: C-28-phosphoniumalkanoyl(C3,C4)- (compounds 5,6); C-3,C-28-betulin-di-TPP conjugates: C-3,C-28-di(phosphoniumalkanoyl(C3,C4)- (compounds 7,8)) were studied. Human platelet-rich plasma (PRP) was used to study a platelets aggregation. Platelet aggregation was measured by Born light transmission technique. The specific aggregation platelets was induced by 1 mM of arachidonic acid.

It was found that betulin caused the decrease of platelets aggregation rate with the highest effect at 10–9 M (turbidity increase amplitude (A) = 52 ± 9%). On the contrary compounds (2, 4–6) promoted platelet aggregation at the concentrations as follows: (2) – 10–5 M; (4) – 10–6 M and 10–5 M; (5) – 10–7 M and 10–5 M; (6) – 10–9-10–6 M. Compounds (1, 7 and 8) induced a decrease of the platelet aggregation likely betulin. The compound (8) was characterized by inhibitory effect on platelet aggregation in a wide range of concentrations (10–9-10–5 M, A = (34 ± 3)% ÷ (79 ± 4)%), whereas the compound (1) and compound (7) had the effect at 10–5 M and 10–9-10–6 M, respectively.

Thus, our experiments showed that betulin and its C-3,C-28-diphosphonium derivatives demonstrated a inhibitory activity on induced aggregation of platelets. We suppose that these compounds can modulate the glucocorticoid receptors which present on the platelet membrane.

This work was funded by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities (4.1493.2017/4.6, 4.5151.2017/6.7) and by Program of Competitive Growth of KFU.

P065-T | Cancer cell growth inhibitory activity of betulin-TPP derivatives and their effect on mitochondria

I. Antipin*,†; O. Tsepaeva†; A. Nemtarev*,†; T. Abdullin*; L. Grigoréva*; E. Kuznetsova*; R. Akhmadishina*; V. Mironov*,†

Kazan Federal University, Kazan, Russian Federation; †A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan, Russian Federation

The ability of some lupane-type triterpenoids and their derivatives to inhibit the growth of tumor cells in vitro and in vivo is well documented. They cause the death of tumor cells mainly through activation of apoptosis via mitochondrial pathway.

A series of triphenylphosphonium (TPP) derivatives of the betulin (C-28-phosphoniumalkanoyl(C1, C3, C4)- (compounds 1,2,3); C-3,C-28-di(phosphoniumalkanoyl(C1, C3, C4)- (compounds 4,5,6)) have been evaluated for their cytotoxic effect against human cancer (MCF-7, PC-3, MCF-7/Vinb) and human skin fibroblast (HSF) cells. Cytotoxicity of tested compounds was evaluated using MTT assay. Changes in transmembrane potential of mitochondria and cell cycle were measured by flow cytometry technique. Betulin possesses a moderate cytotoxicity (IC50 149–233 μM) towards studied cells. The conjugation of betulin with TPP via linker at positions C-28 or C-3, C-28 leads to a decrease of IC50 values. TPP conjugates (1-6) exhibit

high cytotoxicity with IC50 value of 0·045 μM (MCF-7/Vinb). Cytotoxic activity of TPP conjugates of betulin increases in the line: $1 \approx 4 < 3 \approx 6 \approx 5 < 2$. Almost 10-fold difference in cytotoxicity was observed for (2) between HSFs (IC50 2·1 μM) and PC-3 cells (IC50 0·2 μM) as well as between MCF-7 (IC50 0·43 μM) and MCF-7/Vinb (IC50 0·045 μM). Betulin doesn't alter mitochondrial potential in cells in contrast to its derivatives containing phosphonium moiety which induce a decrease in the potential. Mean channel fluorescence of TMRE in treated cells decreased in the range: control (210·8) > betulin (205·9) > compound 3 (186·6) > compound 6 (112·2) > compound 5 (90·1).

So, the compounds with two phosphonium groups affect on mitochondria function more effectively, presumably due to their increased tropism to mitochondria.

This work was funded by the subsidy allocated to KFU for state assignment in the sphere of scientific activities (4.1493.2017/4.6,4.5151.2017/6.7) and by Program of Competitive Growth of KFU.

P066-T | Hyperglycemia and AGEs reduce activation of Tie-2 by Angiopoietin-1 in endothelial cells

R. Sanguineti*; A. Puddu*; M. Nicolò†; G.L. Viviani*

*Department of Internal Medicine and Medical Specialties (dimi), University of Genoa, Genoa, Italy; †Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal-Infant Sciences (DINOGMI), University of Genoa, Genoa, Italy

Endothelial dysfunction is a major characteristic of diabetic microvascular complications. Microvascular changes start in the prediabetic state, become more complex with overt diabetes and remain even when glycemic control is reached. The latest condition is probably sustained by Advanced Glycation End-Products (AGEs), a heterogeneous group of compounds derived from the non-enzymatic reaction of reducing sugars with proteins, lipids or nucleic acids. The angiopoietin growth factor-1 (ANG-1) contributes to vessel integrity by activating the tyrosine kinase receptor (Tie-2). We previously demonstrated that hyperglycemia and AGEs alter the Angiopoietin/Tie-2 system. The aim of the current study was to investigate whether exposure of endothelial cells to hyperglycemia and AGEs may alter angiopoietin-1 signaling in microvascular endothelial cells.

Human microvascular endothelial cell-1 (HMEC-1) were cultured for 5 days with Glycated serum (GS, which consists in a pool of AGEs), 25 mmol/L glucose (HG) or their combination (HG+GS). At the end of the culture, cells were serum starved for 2 hours and stimulated with 200 ng/mL of ANG-1 for 30 minutes. Then we evaluated phosphorylation of Tie-2, Akt and ERK1/2.

Culture in all of the diabetic conditions decreased the ability of ANG-1 to phosphorylate Tie-2. HG reduced both AKT and ERK phosphorylation; whereas AGEs reduced only AKT phosphorylation. Combination of GS with HG had summative effects.

We show that both HG and AGEs negatively alter intracellular signaling induced by Angiopoietin-1 in human microvascular endothelial cells. Since AKT is important for anti-inflammatory action of ANG-1, whereas Erk1/2 is associated with cell migration, our results suggest that AGEs and HG may impair different aspect involved in ANG-1 mediated vascular integrity. These results also highlight the importance of scavenging AGEs to prevent microvascular complications of diabetes.

P067-T | Mesenchymal stem cell transplantation effect on autoimmune encephalomyelitis model

A. Ponomariov*; Z. Gilazieva*; V. Siromiatnikova*; Y. Mukhamedshina*; G. Masgutova*; G. Tezcan*; S. Arkhipova*; S. Khaiboullina*,†; A. Rizvanov*

*Kazan Federal University, Kazan, Russian Federation; †University of Nevada, Reno, Reno, USA

Neuroinflammation is central in pathogenesis of neurodegenerative diseases. Changes in cytokine and neuroprotective molecule are important for predicting the rate of neuron degeneration. Recent studies demonstrated that mesenchymal stromal cells (MSCs) transplantation could be the new therapeutic approach for treatment of these diseases. Study of MSCs effects on neuroinflammation and cytokine activation will help to identify novel biomarkers for potential therapeutics. In this study we sought to determine the serum cytokine profile as well as ultrastructural features of optic and sciatic nerves, spinal cord and brain in autoimmune encephalomyelitis. We also evaluated therapeutic potential of allogeneic MSCs of bone marrow and tooth pulp.

A model of autoimmune encephalomyelitis was established in C57/B16 mice. Allogenic MSCs from bone marrow or tooth pulp were injected intravenously on days 7 and 14 after the disease onset. Serum cytokine profile was analyzed using Bio-Plex Pro Mouse Cytokine 23-plex Assay. Ultrastructures of the optic and sciatic nerves, spinal cord and brain were investigated using transmission electron microscopy (TEM).

The level of proinflammatory cytokines; IL-1a, IL-3, IL-9, IL-12, IL-17, IFN-g, TNF-a, anti-inflammatory cytokine

IL-4 and regulatory chemokines GM-CSF, MIP-1a were significantly increased in all phases of autoimmune encephalomyelitis ($P < 0{\cdot}05$). However, levels of IL-6 and IFN-g levels were increased in the first one and four weeks of the disease, respectively. After MSCs transplantation, IL-1a, IL-3, TNF-a, GM-CSF and IFN-g levels were decreased ($P < 0{\cdot}05$ or ±2-fold difference). According to TEM images, MSCs injections reduced demyelination of neuronal axons.

In conclusion, MSC transplantation affected the serum cytokine levels and nerve ultrastructures in mice with autoimmune encephalomyelitis. Our results provide strong evidence for further investigation of the anti-neurodegenerative properties of MSCs. RAA was supported by state assignment 20.5175.2017/6.7. Work supported by Program of Competitive Growth of KFU.

P068-T | Preventive gene therapy for stroke

V. Markosyan*; F. Bashirov*; M. Sokolov*; F. Fadeev*; A. Izmailov*; M. Kuztetsov*; Z. Safiullov*; A. Rizvanov†; R. Islamov*,†,‡

*Kazan State Medical University, Kazan, Russian Federation; †Kazan Federal University, Kazan, Russian Federation; ‡Kazan Institute of Biochemistry and Biophysics of Russian Academy of Science, Kazan, Russian Federation

The therapy of acute disorders of cerebral circulation is one of the most urgent medical neurologic problems. Unfortunately, the used drugs can slow down some symptoms after ischemic stroke but they are unable to stop the loss of nerve cells leading to disability or death of patients. In this regard, there is a need in developing novel options for protection of the affected neural cells. Recently we have shown the efficacy of cell-mediated gene therapy with genes encoding vascular endothelial growth factor (VEGF), glial cell-derived neurotrophic factor (GDNF) and neural cell adhesion molecule (NCAM) for ischemic stroke in rat model.

In the present study we employed recombinant replication-defective adenovirus serotype 5 (Ad5) carrying VEGF165 and GDNF genes for preventive gene therapy. Virus particles mixture (2×107) of Ad5-VEGF (1/2) and Ad5-GDNF (1/2) was infused intrathecally in 20 μL of saline. Four days after adenoviral-mediated gene delivery brain ischemia was induced by permanent occlusion of the middle cerebral artery (MCA). Animals in the control group were subjected to intrathecal administration of saline.

Evaluation of rat brain cortex three weeks after surgery revealed an infarct zone, which corresponded to the site of MCA occlusion. Morphometric analysis showed the infarct area in rats receiving preventive gene therapy was significantly smaller in comparison with the control animals. Immunofluorescent analysis demonstrated the expression of recombinant VEGF and GDNF in brain cells at the site of stroke.

Thus, intrathecally injected Ad5-VEGF and Ad5-GDNF disseminate by cerebrospinal fluid flow throughout the CNS, transduce neural and glial cells, which increase production of the therapeutic molecules enhancing the survivability of neural cell. This study was supported by the grant of Russian Science Foundation No 17-75-10053. Kazan Federal University was supported by the Russian Government Program of Competitive Growth.

P069-T | Characterization of the multi-unit activity topography in the Rat's Neonatal Barrel Cortex

D. Suchkov*; L. Sharipzyanova*; M. Minlebaev*,†

*Kazan Federal University, Kazan, Russian Federation; †INMED – INSERM U901, Marseille, France

The first postnatal week is a critical period for the somatosensory system and is characterized by the formation of functional somatosensory maps. It is known that there is a progressive tuning of the sensory inputs and their reorganization from diffuse to precise mode (Mitrukhina et. al., 2015). Overlapping of the sensory inputs produces extra complexity for the investigation of the processes underlying development of cortical maps. Here we suggest a convenient approach to characterize the topography of multi-unit activity (MUA) based on the parameters of the sensory evoked response. To test whether differentiation on the non-/topographical inputs could be based on the temporal/frequency properties of the evoked MUA, the imaging and electrophysiological approaches were used. For the neonatal rats (P4-7) localization of two active barrels was done using the optical intrinsic signal (OIS) imaging, followed by the installation of the multisite multishank silicon probe into the detected cortical columns for simultaneous recordings of the activity from the layer 4 of two detected barrels. Analysis of the MUA sensory onset and MUA frequency showed existence of two significantly different clusters with centers at 33 ± 1 ms, 370 ± 70 unit/s and 39 ± 3 ms, 40 ± 20 unit/s. Cortical responses with the characteristics related to the first cluster were predominantly evoked by the stimulation of the topographical sensory input, while adjacent whiskers stimulation resulted in the cortical responses seen predominantly in the second cluster. Thus we propose the clusterization technique that allows the simple definition of the cortical responses evoked by the topographical or non topographical sensory

inputs. This work was supported by RSF grant 16-15-10174 and performed in the frame of the Program of competitive growth of Kazan Federal University.

P070-T | Developmental changes of the multi-unit activity evoked by the single whisker stimulation in the neonatal rat

L. Sharipzyanova*; D. Suchkov*; R. Galimullina*; M. Minlebaev*,†

*Kazan Federal University, Kazan, Russian Federation; †INMED – INSERM U901, Marseille, France

The barrel system is extremely important for rodents. Using whiskers the rodents receive the major part of the sensory information about the external world. The striking feature of neurons in the barrel system is that they respond differently depending on the angular speed and direction of the displaced whisker. While high neuronal sensitivity for whisker displacement was shown in adult barrel system, little is known about the emergence of the velocity sensitivity during development. Here we exploit the evoked cortical multi-unit activity (MUA) to investigate the changes in the evoked cortical response during the critical period of barrel system development (the first postnatal week). Using a combination of the optical intrinsic signal (OIS) and electrophysiological recordings we explored the quantitative parameters of the MUA in the single barrel, received the sensory input from the deflected whisker. Optimized OIS imaging was used to detect the localization of the principal barrel, followed by the positioning of the multisite silicon probe, that allowed us to record electrophysiological activity simultaneously at different cortical depths of one cortical column. We found that during the first postnatal week, there is a progressive increase in the number of the MUA in the granular layer evoked by the whisker shift at the same deflection angle (regression coefficient is $0{\cdot}96 \pm 0{\cdot}07$, $n = 3$, P4-7 rat pups). Variation of the angular speed of deflection didn't affect the developmental phenomena and stronger MUA response was seen in older animals. We suggest that developmental increase of the MUA is associated with the maturation of the whisker-to-barrel pathway and also contributes to the development of the angular tuning and velocity sensitivity in the neonatal barrel system during the critical period of development. The work was supported by RSF grant 16-15-10174 and performed in the frame of the Program of competitive growth of Kazan Federal University.

P071-T | Effect of Nutlin-3a, RG-7112, Carfilzomib and MG-132 on accumulation of p53 in eukaryotic HEK293 cell line

I. Glagoleva*; A. Kudriaeva*; A. Belogurov*,†; A. Rizvanov*; E. Bulatov*

*Kazan Federal University, Kazan, Russian Federation; †Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russian Federation

Tumor suppressor p53 is a well-known transcription factor for multiple genes regulating cell cycle and apoptosis. MDM2 is its negative regulator that binds the p53 transactivation domain and inhibits the ability to activate transcription. MDM2 acts as an E3 ubiquitin ligase that targets p53 for 26S proteasomal degradation. Overexpression of MDM2 is one of the main reasons for impaired functions of the wild type p53. Therefore, it is important to better understand the effect of MDM2 and proteasome inhibitors on molecular and cellular processes in eukaryotic cells. In the reported work we aimed to investigate the effect of p53 activation in HEK293FT cell line using selective MDM2 inhibitors (Nutlin-3a and RG-7112) and 26S proteasome inhibitors (Carfilzomib and MG-132). A range of molecular biology methods were applied including mammalian cell transfection, gel electrophoresis and immunoblotting. The effect of MG-132, Carfilzomib, Nutlin-3a and RG-7112 on the p53 level in HEK293FT cell line was assessed for incubation periods of 24 and 48 hours. We observed that higher inhibitor concentrations lead to a more substantial increase in cellular p53 levels. Particularly, the effect of Carfilzomib and Nutlin-3a on accumulation of p53 was stronger than that of RG-7112 and MG-132. Overall, our results might contribute to the development of novel therapeutics for regulation of p53-mediated processes.

The study was supported by Program of Competitive Growth of Kazan Federal University. Immunoblotting experiments were funded by RFBR research grant 16-34-60213 mol_a_dk.

References:

1. E. Bulatov, A. Zagidullin, A. Valiullina, R. Sayarova, A. Rizvanov. Small molecule modulators of RING-type E3 ligases: MDM and Cullin RING families as targets. Frontiers in Pharmacology, 2018.

P072-T | Artificial microvesicles from human cells: production, biological properties and potential therapeutic use

A. Rizvanov; M. Zhuravleva; L. Tazetdinova; V. Vorobev; S. Kletukhina; S. Kurbangaleeva; O. Neustroeva; M. Gomzikova
Kazan Federal University, Kazan, Russian Federation

Background: The risk of oncological transformation and tumor growth associated with stem cell therapy led to the development of a concept of cell-free therapy, the most promising tool of which are microvesicles – membrane vesicles shed from the cell surface. The main obstacle to the development of pharmaceutical drugs based on microvesicles is limited yield. Here we used the method of cytochalasin B treatment of human cells to increase generation of microvesicles. The purpose of our work was to characterize and evaluate the biological activity of cytochalasin-B-induced microvesicles, or artificial microvesicles (CIMVs), on mesenchymal stem cells (MSC).

Materials and methods: The size of CIMVs was characterized by scanning electron microscopy (SEM, Merlin Carl Zeiss). The angiogenic activity of CIMVs was evaluated by subcutaneous injection in the mixture with Matrigel in Rattus norvegicus. Histological examination of Matrigel implants was conducted 8 days after transplantation.

Results: We found that the size of CIMVs MSC vary from 100 nm to 2600 nm with a peak in the region of 200–1000 nm. After subcutaneous injection in the mixture with Matrigel in vivo CIMVs induced sprouting of $3{\cdot}84 \pm 0{\cdot}16$ blood vessels per mm^2, whereas in negative control (subcutaneous injection of Matrigel) was $0{\cdot}67 \pm 0{\cdot}15$ vessels per mm^2. CIMVs statistically significant (value $P < 0{\cdot}01$) stimulated sprouting of blood capillaries 5·7 times higher than the control sample.

Conclusions: We established that the size of obtained CIMVs is comparable with the size of natural microvesicles. Observed angiogenic activity of CIMVs confirms the perspective of therapeutic application of CIMVs derived from stem and progenitor cells. Pretreating of cells by cytochalasin B increases the yield of CIMVs and makes them perspective pharmaceutic drug.

KFU was supported by Program of Competitive Growth. AR was supported by state assignment 20.5175.2017/6.7 (Ministry of Education and Science of Russian Federation).

P001-F | Redox status of a metastatic microenvironment in the liver of patients with colorectal cancer

K. Usachev*; A.P. Burlaka†; A.A. Burlaka‡; A.V. Vovk†; I.I. Ganusevich†; S. Lukin†,§; S.V. Virko†; K. Iskhakova*; M. Gafurov*
**Kazan Federal University, Kazan, Russian Federation; †R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine, Kyiv, Ukraine; ‡Ukrainian National Cancer Institute, Kyiv, Ukraine; §V.E. Lashkaryov Institute of Semiconductor Physics NAS of Ukraine, Kyiv, Ukraine*

Background: Defects in the mitochondrial electron transport chain (ETC) are considered as the major players in tumorigenesis. Redox state of liver tissues (LT) after the surgery treatment of patients with colorectal cancer (CRC) around the metastasis (Mts) was studied.

Material and methods: LT adjacent to Mts and remote species (5 cm from Mts) from 25 patients with metachronous liver metastases were studied by electron paramagnetic resonance (EPR) with spin-trapping for quantification of the activity of N2 iron-sulfur proteins, levels of NO-N2 complexes, labile iron pool (LIP), lactoferrin (LF), superoxide and NO radicals. Activity of metalloproteinase MMP-2 and MMP-9 were determined by the polyacrylamide gel electrophoresis.

Results: In adjacent and remote LT low activity of N2 in ETC (EPR signal with $g = 1{\cdot}94$), loss of functions of detoxification system (cytochrome P-450, $g = 2{\cdot}25$), appearance and growth of NO-N2 complexes ($g = 2{\cdot}007$) are obtained. Intensive EPR signals from LIP ($g = 2{\cdot}2$–$2{\cdot}4$) and LF ($g = 4{\cdot}3$) are registered. Superoxide generation rates are of up to 5 times higher than in the reference material. NO levels are of 1·7 times higher for the adjacent LT. Activity of MMP-2 and MMP-9 was registered both in adjacent and remote tissues while be higher in 1·7–2·0 times in the adjacent LT.

Conclusions: Formation of the Mts microenvironment in liver is accompanied by superoxide and NO activation of MMP, by the remodeling of the extracellular matrix as well as by the accumulation of LIP and increase of level of LF. Our findings can be used to estimate the functional state of LT with distant metastasis.

Acknowledgments: This work is done according to cooperation agreement between the R.E. Kavetsky Institute and KFU and supported by the Program of competitive growth of KFU.

P002-F | The effects of amitriptyline on the progression of chronic autoimmune inflammation induced by Freund's adjuvant

E. Garanina; V. Khaziakhmetova; O. Muraveva; E. Martynova; L. Ziganshina

Kazan Federal University, Kazan, Russian Federation

Background: It has been long known that some psychotropic drugs exhibit anti-inflammatory properties resulting not from their central psychotropic activity but due to peripheral components. The objective of this study was to investigate potential anti-inflammatory or pro-inflammatory effects of amitriptyline, a classical tricyclic antidepressant, on the model of chronic autoimmune inflammation, adjuvant arthritis.

Material and methods: Experiments were carried out on 20 outbred adult rats with a body weight of 220 ± 10·3 g. An edema was triggered by subplantar injection of 0·1 mL of Freund's adjuvant suspension into the right hind paw of rats. The severity of edema was assessed through measuring paw volume by plethysmometer 37140 (UgoBasile, Italy). Amitriptyline was administered into the stomach once daily for 23 days at 1 mg/kg. The concentration of cytokines (CAV-1, CTGF, IL-6, MCP-1, TNF-a, GRO\KC\INC-1, TIMP-1, tPAI-1, VEGF) was evaluated in rat plasma, and brain and heart tissue homogenates using MILLIPLEX MAP Rat Vascular Injury Magnetic Bead Panel 1 – Toxicity Multiplex Assay.

Results: Long-term monitoring of the intensity of primary inflammatory response revealed that amitriptyline enhanced progression of primary arthritis by 19–33% as compared to control group ($P < 0·05$). However, amitriptyline decreased intensity of progression of secondary arthritis by 54% compared with control group ($P < 0·05$). The effect of amitriptyline was accompanied by a normalization of concentration of VEGF in blood plasma, GRO\KC\CINC-1, VEGF in brain tissues and CAV-1 in heart tissues.

Conclusions: Amitriptyline in the model of chronic autoimmune inflammation of rat paws induced by Freund's adjuvant increased intensity of primary inflammatory response and inhibited progression of secondary arthritis. Effect of amitriptyline leads to normalization of cytokines in plasma, brain and heart of rats.

Work supported by Program of Competitive Growth of KFU.

P003-F | Mitochondrial changes in the maternal liver in a model of obesity (MO) during pregnancy

S.P. Pereira*,†; C.H. Cavallaro*; L. Grilo*; J.D. Martins*; I. Cardoso*; I. Baldeiras*,‡; T. Cunha-Oliveira*; S. Ford§; P.W. Nathanielsz§; P.J. Oliveira*

**CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; †Research Centre in Physical Activity Health and Leisure, Faculty of Sports, University of Porto, Porto, Portugal; ‡Neurological Clinic, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; §Department of Animal Science, University of Wyoming, Laramie, USA*

Background: Pregnancy represents a unique maternal metabolic challenge. Liver disease in pregnancy occurs in 3–10% of cases causing maternal morbidity and mortality. High maternal BMI exacerbates metabolic and hepatic complications. Mitochondrial substrate oxidation supports the maternal and fetal metabolic demands. Mitochondrial defects have been associated with maternal and fetal complications. A better understanding of MO induced maternal physiological changes is needed to prevent adverse outcomes during pregnancy. Our aim was to characterize liver mitochondrial profile and redox network in term pregnant MO ewes.

Methods: Rambouillet:Columbia ewes consumed either an obesogenic (MO: 150% of NRC requirements; $n = 8$), or control diet (C: 100% NRC; $n = 10$) from 60 days prior to conception and through pregnancy. Maternal livers were removed at 0·9 gestation for right lobe measurements. Mitochondrial and antioxidant defense system proteins were determined by Western blot. Mitochondrial respiratory chain complex activities were determined in isolated fractions. Using whole liver tissue we determined catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase activities by spectrophotometry and reduced and oxidized glutathione. Lipid peroxidation was assessed by fluorometry by malondialdehyde (MDA) formation. Data expressed as mean±SE and comparison between groups performed by Mann–Whitney test, P-value <0·05 as significant.

Results: In MO mothers we found increased maternal hepatic MDA indicating greater lipid peroxidation and decreased reduced glutathione, indicating imbalance of endogenous antioxidant defenses. Despite unchanged MO mtDNA copy number, content of proteins implicated in mitochondrial metabolism was altered, with decreased succinate dehydrogenase complex subunit B, increased VDAC1, cyclophilin D and cytochrome c. Complex I activity was decreased in MO-livers.

Conclusion: MO in pregnancy alters maternal hepatic mitochondrial biology impairing redox state, eventually predisposing mothers to metabolic diseases including non-alcoholic fatty liver disease.

Funded by FEDER/COMPETE/FCT-Portugal (PTDC/DTP-DES/1082/2014, POCI-01-0145-FEDER-007440, OCI-01-0145-FEDER-016657, SFRH/BPD/116061/2016 and SFRH/BPD/101169/2014), and NIH (R01HD070096-01A1).

P004-F | Hypoxia-aglycemia induced ischemic responses in the neonatal rat barrel cortex in vitro

E. Juzekaeva*; A. Nasretdinov*; M. Sintsov*; A. Gainutdinov*; M. Mukhtarov*; R. Khazipov*,†

Laboratory of Neurobiology, Kazan (Volga Region) Federal University, Kazan, Russian Federation; †INMED-INSERM UMR1249, Aix-Marseille University, Marseille, France

Anoxic depolarization (AD) is a hallmark of ischemic brain damage. Previously we found that in slices of the rat barrel cortex ischemia-like conditions induced by oxygen-glucose deprivation (OGD) evoked AD, which manifested as a negative LFP shift and an increase in light transmittance and resembles spreading depression (SD). AD typically initiated in one or more barrels and further spread across the entire slice with a preferential propagation through L4. In the present study using simultaneous extracellular local field potential (LFP), optical intrinsic signal (OIS) and whole-cell recordings, we explored the OGD-induced AD in slices of the neonatal (P2-6) rat barrel cortex. We found that OGD-induced ischemic response was not only delayed but also was qualitatively different in the neonatal rats. Ischemic response started with SD-like negative LFP shift associated with transient (~2 minutes) membrane depolarization of ~20 mV at a single-cell level and transient increase in transparency. Transition from SD to AD was characterized by complete but relatively slowly developing neuronal depolarization ~8 minutes after SD without any prominent extracellular LFP signal. Delayed AD was also associated with the second wave of transparency increase during OIS imaging. Thus, in contrast to adult barrel cortex where SD and AD are united, these two processes are dissociated in time in the neonatal cortex. We hypothesize that this developmental differences in the ischemic response involves lower density of voltage-gated channels and synaptic connections, larger extracellular space and lower metabolic demand of immature neurons.

This work was supported by RSF (17-15-01271) and performed in the framework of the Program of Competitive Growth of Kazan Federal University.

P005-F | Novel ischemic penumbra model in vitro

I. Khalilov*,†,‡; A. Vazetdinova*

Laboratory of Neurobiology, Kazan (Volga Region) Federal University, Kazan, Russian Federation; †INMED-INSERM UMR1249, Marseille, France; ‡University Aix-Marseille II, Marseille, France

Stroke is among the leading causes of death and long-term disability of people. Currently in vitro models of stroke involve applying oxygen-glucose deprived (OGD) media over an entire brain slice or plate of cultured neurons. These models fail to mimic the focal nature of stroke as observed clinically. Our aim was to develop a novel in vitro model of stroke that would mimic focal ischemia and thus allow for the investigation of events occurring in the penumbra.

We have used an in vitro preparation of rat intact cerebral cortex fully separated from subcortical structures and the three compartment chamber in which we can put this preparation in three independent compartments. This allows to perfuse the one part of the cortex with OGD medium while bathing the remaining cortex with normal solution. Extracellular field potential and whole-cell recordings and TTC staining were used to characterize OGD-induced response and histological damage.

We found that the OGD-exposed part of the cortex ("core") displayed large transient negative (10–15 mV) DC shift characteristic of the anoxic depolarization and irreversible loss of the membrane potential. Whole-cell recordings from the cortical region adjacent to the OGD-exposed cortex (penumbra) revealed that neurons within this region progressively depolarize throughout one hour of OGD application. Electrophysiological manifestations of the ischemic damage were confirmed using TTC-staining.

Our results suggest that this in vitro model mimics events that occur during focal ischemia in vivo and can be used to study the efficacy of neuroprotectors in the core and penumbra.

This work was supported by RSF (17-15-01271) and performed in the framework of the Program of Competitive Growth of Kazan Federal University.

P006-F | Mitochondrial chaperone is a novel potential player in uterine leiomyoma

M. Grom; M. Makarenko; D. Govsieiev; R. Vorona; V. Berestovyi; I. Sokol; A. Pogribna

Research Institute "Zhyttia", Kyiv, Ukraine

Uterine leiomyoma is the most common indication for hysterectomies worldwide, affecting the reproductive capacity and quality of health of women. The molecular

mechanisms behind the origin of leiomyomas are still relatively unknown. Studies of recent decades emphasize the role of mitochondrial proteins in the development of various diseases. Hsp60 is a mitochondrial chaperone, bur under stress conditions it is actively expressed in extracellular space, where acts as signaling molecule. Serum levels of Hsp60 and anti-HSP autoantibodies changes under various pathologies. The aim of the study was to investigate antibodies to hsp60 in sera of patients with uterine leiomyoma. Therefore, 25 women with uterine leiomyoma before surgery and 6 healthy subjects were recruited to participate in the study. Diagnoses of leiomyoma were confirmed by ultrasonographic and radiographic methods. The recombinant protein generated by Escherichia coli were purified by gel filtration and ion-exchange chromatography. Specific IgG and IgM autoantibodies were measured by ELISA and confirmed in an immunoblotting assay. There were identified no significant difference in levels of IgM antibodies to Hsp60 in sera of women with leiomyoma and healthy donors: 1 of 6 (17%) donors and 3 of 25 (12%) person with uterine leiomyoma had elevated levels of IgM to chaperone. No healthy donors with elevated levels of IgG autoantibodies against Hsp60 were detected, meanwhile 17 of 25 (68%) women with leiomyoma had significantly increased serum levels of autoantibodies to chaperone ($P = 0{\cdot}002$). The data obtained by ELISA were confirmed by western-blot analysis, which indicates the binding of autoantibodies with linear epitopes on the surface of Hsp60. We propose that levels of autoantibodies to Hsp60 may serve as element of panel of protein markers for monitoring of efficacy of treatment of patients with leiomyoma. Further investigation of role of autoantibodies to Hsp60 in pathogenesis may facilitate to personalization of therapeutic strategies.

P007-F | Co-cultivation of moss physcomitrella patens and human skin fibroblasts

E. Zakirova*; I. Chastukhina*; L. Valeeva*; N. Aleksandrova*; A. Palotas*,†; A. Rizvanov*; E. Shakirov*

Kazan Federal University, Kazan, Russian Federation; †Asklepios-Med (Private Medical Practice and Research Center), Szeged, Hungary

Plant tissue represents a promising platform to support animal cell growth in culture as it is less immunogenic and has no known negative impacts on human cell physiology. We aim to develop a new effective natural system based on a living cell alternative to plant-derived cellulose scaffolds for mammalian cell culturing based on live tissue from the model moss Physcomitrella patens. Specifically, we investigated the feasibility of co-cultivation of human skin fibroblasts and P. patens cells in culture and their impact on each other's physiology. We showed that human fibroblast culture grown in the presence of live P. patens protonema at 37°C and 5% CO_2 without light for 30 days retained fibroblast-like monolayer morphology (as visualized by scanning electron microscopy) and surface markers (CD 90, CD 73). We next analyzed the influence of high and low light intensities (880 and 150 lux, respectively) on growth parameters of fibroblasts and moss cells in co-culture. Viability tests showed that proliferation ability of human skin fibroblasts was retained after co-cultivation with moss protonema under low light 150 lux, but not under high light 880 lux. Fibroblast cell culture also did not show any signs of apoptotic and necrotic effects in the presence of P. patens. Thus, our experiments demonstrate the feasibility of co-cultivation of live human and plant cells and the absence of any negative effects on fibroblast growth. Overall, our data indicate that the model moss Physcomitrella patens may indeed be suitable to support mammalian cell culturing in vitro. The work is supported by Russian Foundation for Basic Research grant 18-016-00146a. KFU was supported by Russian Government Program of Competitive Growth.

P008-F | Effect of paclitaxel on ultrastructure of adipose-derived mesenchymal stem cells

L. Tazetdinova*; Z. Gilazieva*; S. Arkhipova*; V. Solovyeva*; E. Alekseeva*; A. Gallyamov†; R. Masgutov*,†; D. Chulpanova*; A. Rizvanov*

Kazan Federal University, Kazan, Russian Federation; †Republican clinical hospital, Kazan, Russian Federation

Current treatment of cancer with drugs is limited due to diverse multidrug resistance acquired by cancer cells and the collateral damage caused to adjacent normal cells by chemotherapy. Mesenchymal stem cells (MSCs) have a tropism toward tumor sites. MSCs are a major component of the tumor microenvironment and play a key role in promotion of tumor progression. Thus, MSCs can be used as vectors to deliver anti-cancer agents. In this study the effect of anti-cancer drug paclitaxel on the morphology of human adipose tissue-derived MSCs (hADSCs) was studied.

Nontoxic concentration of Paclitaxel-Ebewe (PTX) was chosen for hADSC priming. For electron microscopy, hADSCs were incubated with PTX for 24 hours. Ultrastructure of native and primed hADSCs was examined with transmission electron microscope Hitachi HT7700. Native hADSCs demonstrated nucleus of irregular shape and cytoplasm rich in various organelles. Plasma membrane formed protrusions or small size pseudopodia. Incubation of hADSCs with PTX demonstrated significant increase of the cells pseudopodia number in extracellular

space. There was a large number of vacuole-like structures in cytoplasm which resembled droplets. In conclusion, it was shown that hADSCs were not significantly affected by PTX at studied concentrations and this drug can be used for MSCs priming for potential use in targeted anti-cancer therapy. The work was supported by grant from the Russian Foundation for Basic Research 16-34-60201 and Program of Competitive Growth of KFU.

P009-F | Procaine local effects on skeletal muscles in dysferlin-deficient Bla/J mice

O. Chernova*; A. Titova*; M. Mavlikeev*; A. Shafigullina*; A. Zeynalova*; F. Faizrakhmanova*; A. Kiyasov*; R. Deev†,‡

**Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russian Federation; †Human Stem Cell Institute, Moscow, Russian Federation; ‡Ryazan State Medical University named after academician I.P.Pavlov, Ryazan, Russian Federation*

Dysferlin is 230 kDa transmembrane protein involved in repair of sarcolemma. Mutations in DYSF gene lead to dysferlinopathies. Dysferlinopathies are often studied on transgenic mice B6.A-Dysf prmd/GeneJ (Bla/J), that we used to demonstrate regenerative potential of dysferlin after chemical injury by procaine intramuscular injection.

Gastrocnemius muscle of 5 months old Bla/J and C57Bl/6 (control) mice was injected with 100 μL of 0·1% procaine (myotoxic agent). Calf muscles were obtained at 2, 4, 10, 14 days after injection and paraffin sections were stained with H&E, immunohistochemically with antibodies against α-SMA (capillary density), myogenin (terminal myogenic differentiation), Ki-67 (proliferation marker), MHC fast/slow (muscular functional activity).

Necrotic muscle fibers (MF) with leukocytes infiltration were found at all time points after injection with gradual reduction (35·1 ± 9·7% vs 8·7 ± 5·4%, respectively, $P < 0·001$), in C57Bl/6 this parameter was significantly lower. Percentage of centrinucleated MF in Bla/J was significantly lower at 4 days (11·6 ± 1·18% vs 22·5 ± 4·19% in control, $P = 0·03$), remained till 10 days. In Bla/J mice myogenin+ MF maximum was on 4th day after injection (4·4 ± 3·9% vs 9·5 ± 10·01% in C57Bl/6 mice, respectively, $P = 0·046$) but significantly lower at all time points comparing with control, which is an indication of activated but incomplete terminal myogenic differentiation. Capillary density was significantly lower in Bla/J mice only on 4th day (0·15 ± 0·04 vs 0·18 ± 0·07 in control, $P = 0·03$). Proliferative activity was maximal on 2nd day in both groups (13·77 ± 11·08% in Bla/J vs 19·06 ± 19·7% in C57Bl/6, $P = 0·97$) and then decreased till 14th day (0·7 ± 1·09% vs 0·8 ± 1·10%, $P = 0·74$). MHC slow/fast staining demonstrated higher ratio of slow MF in Bla/J in compare with control group at all data point with maximum on 10th day (19·6 ± 22·2% vs 0·07 ± 0·4% in control, $P < 0·001$).

Conclusion: Procaine injection leads to severe myotoxic lesions of Bla/J mice skeletal muscles and regeneration is slower than in control C57Bl/6 mice. Work supported by Program of Competitive Growth of KFU.

P010-F | Contribution of endocrine disruptor pesticides exposures to non-contagious diseases of adult population

N. Stepanova

Kazan Federal University (KFU), Kazan, Russian Federation

A growing body of evidence testifies that endocrine-disrupting chemicals (EDCs) commonly found in the environment contribute to metabolic disorders, especially obesity and diabetes. Information on endocrine disorders caused by pesticides is of special interest. The relationship of indices characterizing the pesticide situation with primary disease incidence and prevalence rate of endocrine diseases (ED, malignant neoplasms (MN), and circulatory diseases in the adult population of the Republic of Tatarstan (RT) for the period from 2000 to 2014 was analyzed. The correlation between morbidity rates and chemical burden was determined with application of Pearson parametric and Spearman non-parametric correlation coefficients. For 15 years, the areas of chemicalization increased by a factor of 2·3, the consumption of pesticides — by a factor of 1·9, that of herbicides — by a factor of 2·85 in RT. The proportion and the volumes of using the glyphosate-containing herbicides increased. The incidence MN and prevalence of circulatory diseases, ED significantly increased among the adult population. The incidence of MN among people of working age increased by 25·7%, among the senior population — even more significantly (by 39·1%; $P < 0·01$). Diabetes mellitus, which was represented by type 2 at the level of 92·5–95·2% in different years, ranked first in the structure of ED. The volume of glyphosate-containing herbicides correlated with primary and total morbidity of MN and prevalence of ED ($r = 0·86$; $P < 0·003$). The indices of using pesticides and mineral fertilizers make 100·0% of direct contribution to prevalence of ED. The pesticides' hazard is limited to intake with foods and drinking water, professional effect, while the impact on the organism of the citizens living on the territory of their use is underestimated. This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities 19.9777.2017/8.9

P012-F | Bacillus pumilus ribonuclease binase induces proinflammatory immune response in macrophages

A. Makeeva*; Y. Surchenko*; H.A. Cabrera-Fuentes*,†; J. Rodriguez-Montesinos†; P. Zelenikhin*; K. Preissner†; O. Ilinskaya*

*Kazan Federal University, Kazan, Russian Federation; †Justus-Liebig-University, Giessen, Germany

Background: Bacterial ribonuclease binase from Bacillus pumilus possess cytotoxic activity against tumor cells. The course of malignancy progression is associated with macrophages migration towards the site of tumor development. NF-κB signaling pathway induce activation of macrophages either into the M1- or into M2- phenotype. Based on previous data from our group on NF-κB signaling pathway in leukemic cells, we propose that binase exhibit cellular activity, including promotion of NF-κB signaling in macrophages.

Material and methods: Binase was isolated as homogenous protein from cultural fluid, enzyme purity was confirmed by electrophoresis. THP-1 and RAW 264·7 cells were obtained from American Type Culture Collection (Rockville, MD). The viability of macrophages was determined using the LDH Cytotoxicity Detection Kit (Roche) and the XTT cell proliferation assay (Life Technologies). Protein expression and cytokine expression were measured by Western Blot and BD Cytometric Bead Array Mouse Inflammation Kit (BD, USA).

Results: We have shown that binase did not decreased macrophages viability. Increased expression of activated NF-κB p65 subunit in macrophages was revealed. Since no changes in MyD88 and TRIF adaptor protein expression were observed, toll-like receptors may not be involved in RNase-related NF-κB pathway activation. In addition, binase induced the release of proinflammatory cytokines IL-6, MCP-1, or TNF-α but not anti-inflammatory IL-4 and IL-10.

Conclusions: NF-κB activation is required by M1 as well as by M2 macrophages differentiation. The M1 phenotype is characterized by the expression of high levels of proinflammatory cytokines, whereas M2 macrophages demonstrate high IL-10 expression. These facts allow us to consider that binase activates macrophages of M1-biased phenotype with tumoricidal properties and stimulates antitumor immunity.

Acknowledgment: The study was performed within the Russian Government Program of Competitive Growth of Kazan Federal University and was supported by the Russian Science Foundation (project no. 14-14-00522).

P013-F | Effects of the homocysteine and its metabolites on oxidative stress and exocytosis of secretory granules in rat pituitary GH3 cells

D. Mishin*; A. Gaifullina*; S. Dmitrieva†; K. Chernova*; V. Atolagbe*; G. Sitdikova*

*Kazan (volga Region) Federal University, Kazan, Russian Federation; †Kazan Institute of Biochemistry and Biophysics-Subdivision of FIC, Kazan, Russian Federation

Endogenous thiols are a group of compounds containing SH-groups and provide cell redox balance. Hyperhomocysteinemia is an increased level of homocysteine in plasma resulted in various pathologies of the nervous system, including developmental disruption in ontogenesis.

The aim of our study was to analysis the effect of homocysteine and its metabolites (homocystine and homocysteine thiolactone) on the oxidative stress and on the basal and evoked exocytosis of secretory granules in rat pituitary GH3 cells. Concentrations of hydroperoxides and malondialdehyde were measured spectrophotometrically. Visualization of cells stained with fluorescent dye FM 1-43 was performed using AxioScope microscope. Cells were incubated in homocysteine, homocystine and homocysteine thiolactone (300 μM) for 20 minutes (acute) and 24 hours (chronic).

It was shown that acute and chronic incubation of cells in all substances induced the increase of hydroperoxide level. At the same time concentration of malondialdehyde, reflected lipid peroxidation significantly increased only by the chronic incubation. Under control conditions 5 minutes incubation of GH3 cells with a fluorescent dye FM 1-43 induced the staining of the membrane, which reflected basal exocytosis. KCl application induced an increase in the intensity of the membrane fluorescence reflected evoked exocytosis. Incubation of cells with homocysteine or homocystine for 20 minutes decreased KCl-evoked and for 24 hours – basal and evoked secretion. Homocysteine thiolactone induces the decrease of basal and evoked exocytosis only after 24 hour incubation period.

It was concluded that homocysteine and its metabolites – homocysteine and homocysteine thiolactone induces oxidative stress in the secretory cells of the pituitary gland and decreases exocytosis of hormone containing granules, results in reduction of growth hormone release. These effects can be implicated in the developmental disruption induced by hyperhomocysteinemia. This work was supported by Russian Science Foundation 14-15-00618 and by Program of Competitive Growth of KFU.

P014-F | Cellular uptake and cytotoxicity of unmodified Pr^{3+}:LaF3 nanoparticles

M. Pudovkin; E. Dudkina; P. Zelenikhin; V. Shtyreva; D. Koryakovtseva; V. Evtugyn; V. Salnikov; A. Nizamutdinov; V. Semashko

Kazan (volga Region) Federal University, Kazan, Russian Federation

Background: Rare earth doped fluoride nanoparticles (NPs) are widely used in biology and medicine. Particularly Pr^{3+}:LaF3 (CPr=7%) NPs demonstrate temperature sensitivity into 20–50°C range (band-shape of the luminescent spectrum strongly depends on temperature). These NPs can be applied in local thermogenesis of living cells and/or in hypothermia. For these applications toxicity of the NPs and features of interaction between the NPs and living cells should be studied thoroughly.

Materials and methods: Pr^{3+}:LaF3 (CPr = 7%) NPs have an average diameter 15 ± 3 nm. Cellular uptake was studied via transmission electron microscopy (Hitachi HT7700 Exalens) at 0·1 g/L NPs concentration using SW 837, A 549, MDCK, LEK, and HuTu 80 cell lines. Toxicity was estimated via MTT assay.

Results: After 1 hour of NPs exposure A 549 and HuTu 80 cells internalized the NPs via micropinocytosis and 100–200 nm agglomerates of the NPs packed into vesicles were found into the cytoplasm. LEK and SW 837 cells did not internalize the NPs which were located onto the external part of the cellular membrane. For MDCK cell culture the NPs were found apart from the cells and internalization did not take place. MTT assay revealed that the NPs are nontoxic into the 0·05–0·5 g/L range. The toxic threshold was found at 1·0 g/L where LEK and A 549 cell cultures demonstrated survivals 68% and 78% respectively.

Conclusions: Pr^{3+}:LaF3 (CPr = 7%) NPs are internalized by some cell cultures. They are nontoxic into the 0·05–0·5 g/L range and hence can be applied in local thermogenesis of living cells and/or hypothermia.

Acknowledgment: The work was funded by the subsidy of the Russian Government [02.A03.21.0002] to support the Program of Competitive Growth of KFU among World's Leading Academic Centers and by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities [3.1156.2017/4.6] and [3.5835.2017/6.7].

P015-F | Changes in the redox status of the brain after low level light therapy

J.C. Bermejo-Millo*; V. García Menéndez†; A. Martín Pernía†; J.A. Martínez Esteban†; H. González Pardo‡; J.L. Arias‡; N.M. Conejo‡; B. Caballero*,§; I. Vega-Naredo*,§; A. Coto-Montes*,§

**Department of Cell Biology and Morphology, University of Oviedo, Oviedo, Spain; †Department of electrical engineering, computer electronics and systems, University of Oviedo, Oviedo, Spain; ‡Laboratory of Neuroscience, Department of Psychology, Instituto de Neurociencias del Principado de Asturias (INEUROPA), University of Oviedo, Oviedo, Spain; §Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain*

Low level light therapy (LLLT) that uses light energy with a near infrared wavelength has received great attention as a new scientific approach with applications in neurology and psychiatry. Currently, there is great uncertainty about the mechanisms of action of LLLT in the brain at the molecular and cellular levels, as well as its possible cytotoxic effects. The objective of this work was to study the alterations related to oxidative stress on different cerebral structures involved in cognitive and emotional functions.

Sixteen adult Wistar rats were divided into four experimental groups and exposed to laser stimulation (20s-long laser pulses, 40s resting time, during 1 hour) with different wavelengths for ten days: Without laser stimulation (Control group), 1064 nm, 905 nm and 650 nm. We evaluated the lipid peroxidation and the total antioxidant activity as tools for assess the redox state after the different exposures in prefrontal, striatum and hippocampus.

We observed that the wavelength of 1064 nm increased lipid peroxidation in the striatum and reduced oxidative damage to lipids in the prefrontal area. Furthermore, this wavelength increased the total antioxidant activity in the striatum and the hippocampus. On the other hand, the wavelength of 905 nm was able to significantly increase lipid peroxidation in the striatum. Finally, the wavelength of 650 nm only impacted on the hippocampus, reducing the total antioxidant activity.

The three types of LLLT produced different changes in the redox status of the brain, in a region-dependent manner. While 1064 nm affected the three regions studied, 905 nm only altered the redox status of the striatum and 650 nm only affected redox status of hippocampus. Our results may support the possible involvement of redox signaling in the LLLT-induced cognitive and emotional effects.

Acknowledgements: This work was supported by PI13/02741, PI17/02009 and GRUPIN-071. PSI2013-45924-P, PSI2015-73111-EXP, PSI2017-83038-P, PSI2017-83893-R, FC-15-GRUPIN14-088.

P016-F | Altered mitochondrial function and oxidative stress in skeletal muscle by leptin deficiency. Attenuation by melatonin

Y. Potes*,†; J.C. Bermejo-Millo*; A. Díaz-Luis*; A. Rubio-González*; B. de Luxán-Delgado‡; J.J. Solano†,§; B. Caballero*,†; A. Coto-Montes*,†; I. Vega-Naredo*,†

**Department of Cell Biology and Morphology, Faculty of Medicine, University of Oviedo, Oviedo, Spain; †Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain; ‡Department for Cancer Stem Cells and Ageing, Barts Cancer Institute, Queen Mary University of London, London, England; §Geriatric Service, Monte Naranco Hospital, Oviedo, Spain*

Physiological decline of leptin levels or leptin resistance results in hyperphagic behavior and nutrient overload, which are the central features of eating disorders. Skeletal muscle has an efficient capability to cover energy expenditure modifications by the effect of endocrine axes, such as leptin signaling. Thus, a direct metabolic regulation by leptin should be in skeletal muscle. The aim of this study was to evaluate the effect of leptin deficiency in mitochondrial bioenergetics and its implication on skeletal muscle quality. Furthermore, as leptin and melatonin are two hormones that exhibit circadian rhythms and have a major influence on energy balance and the regulation of body mass, one can ask whether melatonin treatment could mimic leptin effects on skeletal muscle fibers.

Sixteen six-week-old male wild-type (C57BL/6J) and sixteen six leptin-deficient ob/ob (B6.V-Lepob/J) mice (Charles River Laboratories España SA, Spain) were housed under 12:12 hours dark-light cycle with standard chow diet and tap water ad libitum. Half mice of each experimental group were subcutaneously injected with 500 μg melatonin/kg for four weeks at ZT14.

Our findings showed that ob/ob mice presented alterations in electron transport chain (ETC) machinery and mitochondrial function. Leptin-deficiency, due the constant feeling of limited energy supplies, forces skeletal muscle to enhance oxidative phosphorylation, resulting in an excess of energy production and the activation of the pro-oxidant p66Shc signalling pathway together with a poor mitochondrial antioxidant capability. It culminates in higher lipid peroxidation and a decline of muscle quality. Melatonin treatment plays a significant role in regulating mitochondrial energy homeostasis that lead to an adaptation on the redox system by reducing lipid peroxidation and increasing antioxidant activities.

Therefore, melatonin could be a potential therapeutic agent for leptin-related disorders by mimicking leptin signaling.

Acknowledgements: This work was supported by PI13/02741, PI17/02009 and GRUPIN-071. Y.P. is a FISS predoctoral fellow (FI14/00405).

P017-F | Alternate assembly of SDHA regulates energy balance under bioenergetic stress

L. Krobova*; K. Vanova*; A. Bezawork-Geleta†; H. Wen‡; M.P. Molloy§; S. Boukalova*; R. Zobalova*; S.M. Novais*; P. Abaffy*; R. Naraine*; L. Dong†; K. Pacak¶; J. Rohlena*; S. Park‡; J. Neuzil*,†

**Institute of Biotechnology, Czech Academy of Sciences, Prague, Czech Republic; †School of Medical Sciences, Griffith University, Southport, Australia; ‡College of Pharmacy, Natural Product Research Institute, Seoul National University, Seoul, Korea; §Australian Proteome Analysis Facility, Macquarie University, North Ryde, Australia; ¶Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, USA*

Introduction: Mitochondrial complex II (CII) A.K.A succinate dehydrogenase (SDH), comprising four protein subunits SDHA-D, directly links tricarboxylic acid cycle and oxidative phosphorylation (OXPHOS). As such, CII is in a position to regulate these two systems crucial for energy production and essential metabolite synthesis. In the present work we therefore investigated if CII can orchestrate adaptive responses to bioenergetic stress induced by mitochondrial DNA (mtDNA) depletion or by the loss of CII subunit SDHB.

Methods: MtDNA-depleted cells (derived from murine 4T1 and human MCF7 breast cancer cells) and SDHB KO cell lines (derived from MDA-MB-231 triple-negative breast cancer cell line) were used for the experiments. Permeabilized cells, mitochondria and whole cell lysate were used for respiratory assay, native blue gel electrophoresis (NBGE) and western blotting, respectively. High throughput analysis (SWATH-MS, RNAseq and metabolomics) was used to evaluate de novo pyrimidine synthesis. Immunocompromised Balb-c nu/nu mice were used for xenograft experiments.

Results: Both depletion of mtDNA and knock-out of SDHB had an unexpected effect on CII assembly resulting in high levels of an alternative species we refer to as CII(low). CII migrated on NBGE at 100 kDa (as opposed to the 130 band for mature CII), containing SDHA associated with selected CII assembly factors. The presence of CII(low) in cells introduced systemic changes leading to the attenuation of energy-intensive anabolic processes such as pyrimidine biosynthesis to maintain energy balance. This effect was partially reverted by the depletion of CII(low) following SDHA knock down in SDHB-deficient cells. The resulting imbalance was emphasized by the failure of SDHB-deficient cells lacking CII(low) to form tumors in mice.

Conclusions: We conclude that CII(low), induced by mtDNA depletion and/or deficiency of CII subunits plays an important role in the homeostatic control of metabolite synthesis under bioenergetics stress.

P018-F | Acute changes in cortical network functions during endothelin-1 induced local brain ischemia

D. Vinokurova*; A. Zakharov*; N. Lotfullina*; G. Burkhanova*; K. Chernova*; J. Lebedeva*; R. Khazipov*,†

Laboratory of Neurobiology, Kazan Federal University, Kazan, Russian Federation; †INMED-INSERM UMR1249, Aix-Marseille University, Marseille, France

Subarachnoid hemorrhage is often complicated by cerebral vasospasm and delayed ischemic damage. Key player in this process is a potent vasoconstrictor endothelin-1 (ET-1). In the present study, we explored the effects of epipial application of ET-1 on spontaneous and sensory-evoked activity at different depths of the cortical column of rat barrel cortex (whiskers representation area) using linear 16-channel silicon probes. ET-1 was delivered epipially for 1 hour and then washed by ACSF during 3 hours. ET-1 application first caused several minutes long suppression of both spontaneous and evoked activity in all cortical layers, followed by a short burst of multiunit activity organized in gamma oscillation in layers 5 and 6 and then by cortical spreading depression (CSD). In most cases spontaneous and sensory-evoked activity was fully blocked after CSD episode and remained severely depressed during ET-1 application. In some cases, activity partially recovered in deep layers after the first CSD and several recurrent CSDs restricted to the deep layers were observed. In all cases slow large negative shift of the extracellular potential developed through the time course of ET-1 application attaining maximal values of up -80 mV in deep layers. Washing of ET-1 resulted in recovery of the DC potential, but only weak recovery of spontaneous and evoked activity was observed. Histological examination of brain sections revealed focal ischemic damage in the barrel cortex at the site of ET-1 application. Thus, ET-1 induced focal ischemia induces sequential cortical layer specific changes in spontaneous and sensory-evoked activity in cortex that could be useful for the rapid detection of the onset of cerebral vasospasm and ischemia and for the prevention of the delayed ischemic damage in patients with subarachnoid hemorrhage.

P019-F | Role of Parkin in the age-mediated decline in brown adipose tissue thermogenic activity

M. Cairó*,†,‡; A. Delgado-Anglés*,†,‡; L. Campderrós*,†,‡; F. Villarroya*,†,‡; M. Giralt*,†,‡; J. Villarroya*,†,‡,§

Universitat de Barcelona, Barcelona, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición, Barcelona, Spain; ‡Institut de Biomedicina Universitat de Barcelona (IBUB), Barcelona, Spain; §Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Background: Aging leads to a decline in brown adipose tissue (BAT) activity, underlying increased propensity to metabolic diseases and obesity in elderly individuals. Selective autophagic degradation of mitochondria (mitophagy) is a quality control mechanism for maintaining a healthy mitochondrial pool, and the E3-ubiquitin ligase Parkin is a key component of this process. We described previously that autophagy is repressed during BAT thermogenic activation. Here, we analysed the specific role of Parkin in BAT function.

Material and methods: To analyse the role of Parkin in BAT activity, we exposed wild-type and Parkin-KO mice to distinct challenges affecting BAT activity: environment temperature, diet and age. We analysed the impact of Parkin deficiency in metabolic parameters and BAT thermogenic markers. For in vitro assays we used primary cultures of brown adipocytes.

Results: We found that Parkin expression is repressed during cold-induced thermogenesis (BAT activation) and induced during cold-deacclimation (BAT inactivation associated with a massive loss of mitochondrial protein). Accordingly, Parkin-KO mice had an enhanced thermogenic activity of BAT and were resistant to the age and diet-induced obesity. The absence of Parkin prevented the degradation of mitochondrial proteins and the reduction of mitochondria DNA during the inactivation of BAT occurring in cold-deacclimation and aging. Aberrant mitochondria appeared in BAT from Parkin-KO mice in these conditions. Moreover, the naturally-occurring rise in FGF21 levels associated with aging is abolished in Parkin-KO mice, in association with specific impairment of FGF21 expression in adipose tissues. The aging-associated decline in the expression of the FGF21 co-receptor β-Klotho in BAT is prevented in Parkin-KO mice.

Conclusions: Parkin appears to be a key factor for the regulation of the mitochondria quality and mass, and thermogenic function in BAT. Parkin is also involved in the control of FGF21 release and signalling in BAT in association with aging.

P020-F | Mitochondriotropic antioxidants based on dietary phenolic acids as modulators of oxidative stress on skin fibroblasts

C. Veloso*; J. Teixeira*,†; F. Cagide†; S. Benfeito†; C. Oliveira†; F. Borges†; P. Oliveira*

Center for Neurosciences and Cell Biology, UC Biotech Building, Lot 8A, Biocant Park 3060-197 Cantanhede, PORTUGAL, Coimbra,

Portugal; †CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Background: Age-related skin structural changes involves disruption of mitochondrial function in that organ. Importantly, mitochondria are critical organelles which besides participating in energy production, can generate oxygen reactive species (ROS) and trigger pro-apoptotic signals. Some ROS can damage mitochondria and disturb their functional capacity. Polyphenols such as hydroxybenzoic (HBA) and hydroxycinnamic (HCA) acids antioxidant properties have been extensively studied although their utility against skin aging is still limited. So, the protective effects of novel mitochondriotropic antioxidants based on HCA and HBA were studied on oxidative stress-induced models in skin fibroblasts.

Material and methods: Normal human dermal fibroblasts (HDF) were used to test the toxicological profile of novel mitochondriotropic antioxidants, by measuring cell viability and metabolic parameters, as well as their protection in in vitro models of oxidative stress.

Results: The novel mitochondriotropic antioxidants showed dose-dependent cytotoxic effects that were only relevant for concentrations above those where antioxidant activity was observed. MitoQ and AntiOxCIN6 were shown to be the most toxic of all studied. The other mitochondria-targeted antioxidants showed much lower toxicity. Toxicity profiles and protection against oxidative stress-induced cytotoxicity on HDF were compared with those of natural antioxidants, e.g. caffeic, rosmarinic and gallic acids, and resveratrol, as well as with MitoQ, a commercially-available mitochondria-directed antioxidant. AntiOxBEN2 and AntiOxCIN4 showed a better safety profile when compared with the latter compound.

Conclusions: New insights into the performance of mitochondriotropic antioxidants based on dietary scaffolds as modulators of mitochondrial oxidative stress has been accomplished. Given the increased need for solutions to target skin aging, the novel molecules may be used as anti-skin aging active ingredients in topical skin products.

Funded by FEDER funds through the Operational Programme Competitiveness Factors – COMPETE and national funds by FCT – Foundation for Science and Technology under research grants PTDC/DTP-FTO/2433/2014, POCI-01-0145-FEDER-016659, POCI-01-0145-FEDER-007440, POCI-01-0145-FEDER-006980, NORTE-01-0145-FEDER-000028. C. Veloso (PTDC/DTP-FTO/2433/2014).

P022-F | A high-throughput method for catalase activity determination

L. Grilo*; C.H. Cavallaro*; J.D. Martins*; P.J. Oliveira*; S.P. Pereira*,†

**CNC – Center for Neuroscience and Cell Biology, UC-Biotech, Biocant Park, University of Coimbra, Coimbra, Portugal; †Research Centre in Physical Activity Health and Leisure, Faculty of Sports, University of Porto, Porto, Portugal*

Background: The development of rapid, reliable and highly reproducible biological assays that can be standardized and routinely used in preclinical tests or clinical diagnostics constitutes a powerful strategy to decrease the financial burdens and time consumption characteristic of the normal pipelines of drug discovery or diagnose. Hydrogen peroxide (H_2O_2) is deleterious for cells at high concentrations, while at physiologic levels it is important in cell homeostasis and regulates different cellular and molecular signaling mechanisms. The vital balance of H_2O_2 levels is achieved by enzymes such as catalase and glutathione peroxidase and by the involvement of specialized cellular organelles such as the peroxisomes. Catalase is present in mostly all aerobic cells and is a major responsible for H_2O_2 degradation. The determination of catalase activity can be useful to assess a tissue or cell's response to stress. Our aim is to develop a rapid and high-throughput method to assess catalase activity in tissues and cell samples.

Results: We developed a method to determine catalase activity in a 96-wells plate format. Our procedure allows the simultaneous measurement of catalase activity in multiple samples from cells and tissues, the use of small sample amounts (0·25–2 μg) and integrates different positive and negative controls which are run simultaneously, thus eliminating the variations usually arising from running separated assays. The developed procedure is less time-consuming and drastically reduces the amount of sample and all other reagents required by the conventional tests available for catalase activity determination. Despite of this increased efficiency, the robustness of catalase activity determination is maintained and, therefore, the proposed method allows the generation of results with a high degree of confidence.

Conclusion: We established a highly-sensitive, simple, rapid, direct and cost-effective bioassay for measuring catalase activity in a variety of biological samples.

Funded by FEDER/COMPETE/FCT-Portugal (PTDC/DTP-DES/1082/2014, POCI-01-0145-FEDER-007440, OCI-01-0145-FEDER-016657, SFRH/BPD/116061/2016).

P023-F | The bittersweet relation between O-GlcNAcylation and Alzheimer's disease: a focus on mitochondria

T.S. Pinho*; S.C. Correia*,†; P.I. Moreira*,‡

*Center for Neuroscience and Cell Biology (CNC), Coimbra, Portugal; †Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; ‡Laboratory of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Uncover the initial cause(s) underlying Alzheimer's disease (AD) pathology is imperative for the development of new therapeutic interventions to counteract AD-related symptomatology and neuropathology in a timely manner. The early stages of AD are characterized by a brain hypometabolic state as denoted by faulty glucose uptake and utilization and abnormal mitochondrial function and distribution which, ultimately, culminates in synaptic "starvation" and neuronal degeneration. Importantly, it was recently recognized that the post-translational modification O-GlcNAcylation (O-GlcNAc) modulates mitochondrial function, motility and distribution being proposed to act as a nutrient sensor that links glucose and the metabolic status to neuronal function.

Using post-mortem human brain tissue, in vivo (triple transgenic mouse model of AD (3xTg-AD)) and in vitro models of AD (differentiated SH-SY5Y cells exposed to AD-mimicking conditions), the present study is aimed to clarify whether O-GlcNAc contributes to "mitochondrial pathology" in AD and its potential as a therapeutic target.

A reduction in global O-GlcNAc levels was observed in the brain cortex and hippocampus of AD subjects. In accordance, O-GlcNAc levels were also diminished in the brains of 3xTg-AD mice. In vitro models of AD also exhibited a marked reduction in global O-GlcNAc levels, which was strongly correlated with hampered mitochondrial bioenergetic function and disruption of the mitochondrial network and fusion-fission balance. Consequently, an alteration in the protein levels of synaptic integrity markers and loss of cell viability were also observed. Conversely, the pharmacological modulation of O-GlcNAcylation levels with Thiamet-G restored O-GlcNAc levels, cell viability and synaptic integrity in in vitro models of AD.

Overall, these results suggest that O-GlcNAc is in the basis of AD pathology functioning as a potential link between mitochondrial energetic crisis and synaptic and neuronal degeneration, and thus representing a promising therapeutic target to tackle this devastating neurodegenerative disease.

P024-F | Effectiveness of local hypothermia in the infarct-related artery to reduce reperfusion injury of the myocardium

S. Zinchenko*; A. Khalirakhmanov*; A. Sharafeev*; J. Troshina†

*Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation

Background: The damage from reperfusion injury that occurs directly during the restoration of blood flow in ischemic tissue after PCI can be up to 50% of the final infarct size. Restoration of tissue blood supply with minimization of reperfusion injury is required for qualitative perfusion of the myocardium and can be achieved by cardioprotection during reperfusion. The effectiveness of local hypothermia of the Central artery to reduce reperfusion damage to the myocardium was evaluated.

Materials and methods: We treated 21 patients with STEMI (LAD TIMI-0), which underwent primary PCI with local hypothermia in the infarct-related artery. The control group (CG) included of 30 patients, with the same localization of the STEMI which was performed the standard PCI. Men had 67% MGvs. 62% CG. Diabetes mellitus: 7% – in the MG, 9% – in the CG. Regional hypothermia was performed by an intracoronary solution of 0·9% NaCl cooled to +7°C at a rate of 15 mL/min. Reperfusion arrhythmias and ST segment changes were assessed during the first day, viable myocardial volume (MRI) after 6 months.

Results and discussion: Reperfusion arrhythmias were detected in 14·3% in the MG and 33·3% in the CG ($P < 0{\cdot}05$), Ventricle arrhythmia were 14·3% in the MG and 16·5% in the CG. ST elevation V1-V5 more than 1500 μV, stenosis of the left main coronary artery, smoking, history of AMI, therapy with aspirin, β-blockers, and statins were an independent predictors of reperfusion arrhythmias. The size of the final zone of necrosis (MRI data) was 23 ± 7% in the MG and 32 ± 9% in the CG ($P < 0{\cdot}05$).

Conclusion: Local hypothermia of the infarct-related artery allows to reduce the reperfusion arrhythmias (14·3% vs 33·1%) and zone of myocardial necrosis (23 ± 7% in the MG and 32 ± 9% in the CG ($P < 0{\cdot}05$).

Work supported by Program of Competitive Growth of KFU.

P025-F | Analysis of cytokines level in mice with amyotrophic lateral sclerosis after mesenchymal stromal cell infusion

A. Mullakhmetova; Z. Gilazieva; V. Syromiatnikova; E. Garanina; G. Masgutova; Y. Mukhamedshina; G. Tezcan; S. Arkhipova; A. Rizvanov

Kazan Federal University, Kazan, Russian Federation

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. One of the pathological hallmarks of ALS is neuroinflammation. Therefore, developing therapeutic tools to reduce neuroinflammation is important. Several studies demonstrated that by migrating to the area of inflammation, mesenchymal stromal cells (MSCs) can reduce the extent of systemic inflammatory response. In this study, we aimed to investigate the effect of different type of MSCs on proinflammatory cytokine levels in serum, spinal cord and brain of transgenic ALS mice.

Materials and methods: Transgenic SOD1-G93A mice (genotype B6SJL-TG(SOD1-G93A)dl1Gur/J) were intravenously injected with different types of MSCs (derived from adipose tissue (ADMSC) and tooth pulp (TPMSC) 7 and 14 days after the onset of first symptoms. Cytokine profile of blood, spinal cord and brain samples were analyzed using Bio-Plex Pro Mouse Cytokine 23-plex Assay (Bio-Rad).

Results: We found that the IL-1a, IL-3, IL-9, IFN-g, Eotaxin, GmCSF, KS, MCP1b, PANTES were up-regulated in the brain after ADMSC transplantation. In groups with TPMSC transplantation, the IL-4 and IL-5 values were increased ($P < 0{\cdot}05$). Moreover IL-1a, IL-1b, IL-3, IL-9, IL-12 were down-regulated in blood serum and G-CSF, KC, MIP-1a, MIP-1b, RANTER in spinal cord.

Conclusions: Our results suggest that MSCs transplantation have an immunomodulatory effect on inflammation by modifying level cytokines in serum, brain and spinal cord of ALS animals. RAA was funded by state assignment 20.5175.2017/6.7 (Ministry of Education and Science of Russia). This work was supported by Program of Competitive Growth of KFU.

P026-F | Effect of mesenchymal stromal cell transplantation on pro-inflammatory cytokine pattern in transgenic mice with Alzheimer's disease

A. Mullakhmetova; Z. Gilazieva; V. Syromiatnikova; E. Garanina; G. Masgutova; Y. Mukhamedshina; G. Tezcan; S. Arkhipova; A. Rizvanov

Kazan Federal University, Kazan, Russian Federation

Background: Alzheimer's disease (AD) is the most common form of dementia. The pathology of AD is associated with accumulation of abnormal protein aggregates, neuroinflammation and activation of microglia and astrocytes. Treatment of AD includes inhibition of inflammation to elevate symptoms of the disease. Recent studies demonstrated that mesenchymal stromal cells (MSCs) can reduce the extent of systemic inflammatory response by migrating to the area of inflammation and providing immunomodulatory support. However, exact mechanisms of anti-inflammatory effects of MSCs in AD patients remain unknown. Therefore, in the present study, we aimed to investigate the effect of adipose, bone marrow and tooth pulp derived MSCs transplantation on cytokine levels in blood and brain in murine AD model.

Material and methods: Transgenic APP/PS1 mice (genotype B6C3 – Tg(APP695)85Dbo Tg(PSENI)85Dbo) were intravenously injected with different types of MSCs (derived from adipose tissue, bone marrow or tooth pulp) 7 and 14 days after onset of first symptoms. Cytokine profile of blood and brain samples were analyzed using Bio-Plex Pro Mouse Cytokine 23-plex Assay (Bio-Rad).

Results: Our results indicate that the onset of the disease is characterized by decreased level of proinflammatory cytokines IL-1a and TNF-a decrease and increased level of the anti-inflammatory cytokine IL-5 in serum samples. Additionally, increased GM-CSF and decreased RANTES levels were found in serum of these mice. Also, IL-1b, IL-3, G-CSF and KC levels were significantly lower of GM-CSF in brain homogenate of MSCs treated groups. There were no significant differences in cytokine levels after injection of different types of MSC ($P < 0{\cdot}05$ or $\pm$2-fold difference).

Conclusion: MSCs transplantation reduced inflammation though modifying cytokine levels in AD mice. RAA was supported by state assignment 20.5175.2017/6.7 Ministry of Education and Science of Russian Federation. Work supported by Program of Competitive Growth of KFU.

P027-F | Aging is associated with alterations of intestinal microbiota composition and barrier function

A. Brandt*; A. Camarinha-Silva†; C.J. Jin‡; A. Baumann*; A.J. Engstler*; A. Nier*; C. Sellmann‡; C. Schmeer§; O.W. Witte§; I. Bergheim*

**University of Vienna, Vienna, Austria; †University of Hohenheim, Stuttgart, Germany; ‡Friedrich-Schiller-University Jena, Jena, Germany; §University Hospital Jena, Jena, Germany*

Background: Among many other factors, intestinal homeostasis determined by intestinal microbiota composition and

barrier function also seems to play an important role for health and well-being during aging. The aim of the study was to determine if the increased bacterial endotoxin frequently found in aged humans and rodents and thought to be involved in inflamm-aging is associated with impaired intestinal barrier function and modified microbiota composition.

Material and methods: Bacterial endotoxin level in portal plasma, markers of intestinal barrier function and the intestinal nitric oxide system were assessed in 3 and 24 months old healthy male C57/Bl6 mice fed standard diet. Illumina amplicon sequencing was used to determine microbiota composition and diversity.

Results: Endotoxin concentration in portal vein of 24 months old mice was significantly higher than in young mice. While histology and number of goblet cells was not altered, old mice showed lower levels in occludin and zonula occludens-1 tight junction protein and mRNA concentration in proximal small intestine, but not in ileum or colon. The loss of tight junction proteins in this proximal part of small intestine was associated with lower levels of lysozyme and cathelicidin-related antimicrobial peptide mRNA expression as well as 3-nitrotyrosine protein adducts, inducible NO-synthase mRNA expression and NO2- as well as lower L-citrulline concentration in plasma of portal vein. Moreover microbial diversity was significantly lower in 24 months old mice and abundance of phylum Bacteroidetes by trend was lower whereas abundance of Firmicutes was higher compared to young mice.

Conclusion: Our data suggest that old mice despite appearing healthy and being fed standard chow suffer from impaired intestinal barrier function associated with altered nitric oxide system and altered intestinal microbiota composition in small intestine. (Funded by DFG FKZ: BE2376/8-1)

P029-F | Novel dual binding site inhibitor for treatment of Alzheimer's disease

I. Zueva; V. Semenov; K. Petrov; E. Nikolsky

Institute of Organic and Physical Chemistry Subdivision of the Federal State Budgetary Institution of Science "Kazan Scientific Center of Russian Academy of Sciences", Kazan, Russian Federation

Background: Currently inhibition of acetylcholinesterase (AChE) is a main strategy for treating Alzheimer's disease (AD), but no AChE inhibitors have yet been shown to slow the progression of AD. AChE itself promotes the formation of Aβ plaques because of interaction between Aβ and the peripheral anionic site (PAS) of the enzyme. In our previous work we showed that the effective dose (5 mg/kg, i.p.) of PAS inhibitor compound 3d (6-methyluracil derivative) can simultaneously improve cognition and slow down the rate of Aβ aggregation (Semenov et al., 2015).

Material and methods: To check whether 6-methyluracil derivatives slow the progression of the disease by reducing the amount of Aβ deposits APP/PS1 transgenic mice were treated with compound 3d (5 mg/kg, i.p) once a day for a total of 18 successive days and trained on a reward alternation task using a T-maze. After behavior experiment the number of Aβ deposits and the synaptic density in the cerebral cortex were analyzed.

Results: Compound 3d significantly improved the percentage of reaching behavioral criterion in T-maze task, reduced number of Aβ deposits and prevented synaptophysin density loss in brain of APP/PS1 mice.

Conclusions: The ability 6-methyluracil derivatives to reduce the rate of Aβ aggregation can be considered as an additional mechanism, which will help to decrease the rate of memory loss in AD.

This work was supported by Russian Science Foundation grant 14-50-00014.

P031-F | Effect of etomoxir on mitochondrial function assessed by high-resolution respirometry

F.S.G. Silva*; T. Komlodi†; L.F. Garcia-Souza†; P.J. Oliveira*; E. Gnaiger†,‡

**Center for Neuroscience and Cell Biology, Coimbra, Portugal; †Oroboros Instruments, Innsbruck, Austria; ‡Department Visceral, Thoracic Surgery, Medical University of Innsbruck, Innsbruck, Austria*

Background: Non-alcoholic steatohepatitis is related to liver mitochondrial dysfunction; therefore, improved protocols are developed to evaluate mitochondrial fitness in the course of the disease. To modulate respiration during fatty acid oxidation (FAO), etomoxir, inhibitor of carnitine palmitoyltransferase-I, is often used (40–400 μM) including in commercial kits to block mitochondrial fatty acid (FA) transport. We investigated the specific effect of etomoxir on FAO in comparison to unspecific inhibitory side effects on mitochondrial respiration.

Methods: We tested 40 and 200 μM etomoxir using high-resolution respirometry on permeabilized Huh7 human hepatocellular carcinoma cells and mitochondria isolated from mouse liver and brain mitochondria. We used substrate-uncoupler-inhibitor titration (SUIT) protocols to analyze OXPHOS (P) and electron transfer (E) capacities of the FA-, NADH- and succinate pathways separately (F, N, S) and in combination (FN, FS, FNS, NS). The F-, N- and S-pathways were studied separately or in combination using

palmitoyl-carnitine + malate, or pyruvate + glutamate + malate, or rotenone + succinate, respectively.

Results: 200 μM etomoxir inhibited not only F-capacity, but all mitochondrial core metabolic pathways were also significantly impaired. This is the case even in brain mitochondria where F-capacity is extremely low compared to liver. While 200 μM etomoxir inhibits significantly N- and S-pathways, 40 μM etomoxir showed a non-significant inhibitory trend towards N- and S-pathways. These results indicate an inhibitory effect of etomoxir on downstream of the Q-junction.

Conclusions: The inhibitory pattern observed in the SUIT protocols suggests an unspecific effect of etomoxir on mitochondrial respiration involving N-and S-pathways and raises caution in its application as specific inhibitor of FAO.

Acknowledgement: The mtFOIE GRAS project received funding from the European Union's Horizon-2020 Research and Innovation program under the Marie Skłodowska-Curie Grant Agreement No. 734719. Filomena Silva is recipient of a Post-Doctoral Fellowship from the Foundation for Science and Technology, SFRH/ BPD/ 122648/2016.

P032-F | Sirtuins 1 and SIRT 3 in brain injury after chronic cerebral hypoperfusion in a mouse model

T. Lapikova-Bryhinska*; O. Harmatina*,†; T. Voznesenskaya*; N. Grushka*; O. Shepel*; I. Kolosova‡; L. Bratus*

Bogomoletz Institute of Physiology of NAS of Ukraine (BIPH), Kyiv, Ukraine; †Institute of Neurosurgery named after acad. A.P.Romodanov of the NAMS of Ukraine, Kyiv, Ukraine; ‡National Military Medical Clinical Center Main Military Clinical Hospital, Kyiv, Ukraine

Background and Purpose: Sirtuin 1 and 3 are members of the silent information regulator family of proteins. Both of them have been shown to play roles in brain aging and neurological disorders. The purpose of this study was to determine of the gene expressing SIRT1 and SIRT 3 effects in brain tissue during the chronic cerebral hypoperfusion.

Methods: Adult male C57BL/6J mice were treated by intraperitoneal injection with nicotinamide (1 group – 200 mg/kg, 10 days after UOCCA) and with resveratrol (2 group – 20 mg/kg, 7 days to and 3 days – after UOCCA) after cerebral hypoperfusion induced by unilateral occlusion of the left common carotid artery (UOCCA). Results of Doppler ultrasound of common carotid arteries, DNA damage detected by the Comet assay (also known as the single cell gel electrophoresis assay) and the levels SIRT1 and SIRT3 gene expression were estimated by real-time PCR analysis (RT-PCR) in brain tissue were assessment performed at 2 months after UOCCA.

Results: The results of the Comet assay showed increase of the brain cells number with DNA breaks in mice with UOCCA. We found that administration of nicotinamide and resveratrol decrease in the percentage of brain cells DNA damage in UOCCA induction. The expression of gene SIRT1 was also changed in all groups. Showing the increase levels of SIRT1 and SIRT3 mRNA in UOCCA groups with resveratrol and nicotinamide injections. The resulting flow changes in the right carotid artery was revealed by Doppler US and was higher compared with the control group.

Conclusions: Thus, our results indicate that chronic cerebral hypoperfusion induces the brain tissue damage and SIRT1 and SIRT3 can participate in neuroprotective mechanisms under that conditions, and resveratrol and nicotinamide may be promising in the treatment of brain ischemic damage.

P033-F | Hepatic mitochondrial dysfunction induced by silver nanoparticles in Sprague-Dawley rats

R. Silva*; J. Teodoro*; A. Varela*; F. Duarte*; A. Rolo*; S. Hussain†; C. Palmeira*

Department of Life Sciences of the Faculty of Sciences & Technology of the University of Coimbra, Coimbra, Portugal; †711th HPW/RHDJ, Molecular Bioeffects Branch, Bioeffects Division, Human Effectiveness Directorate, Air Force Research Laboratory, Wright Patterson AFB, Dayton, USA

Background: The use of nanoparticles (NPs) has been steadily increasing worldwide due to their fascinating and particular physicochemical properties that wildly differ from the same materials, on a larger scale. From all types and shapes of NPs, silver NPs (AgNPs) are by far the most ubiquitous type of nanomaterial. Due to their probable harmful effects to both humans and the environment, a great number of studies have been conducted on the potential for injury by NP exposure. However, despite several indications of possible harmful effects, no long-term studies using a low dose of AgNPs have been conducted in vivo.

Material and methods: Sprague–Dawley rats were weekly intraperitoneally injected with either 10- or 75-nm AgNPs, with or without a previous injection of the antioxidant N-acetylcysteine (NAC), for 4 weeks. Animals were sacrificed and various parameters (including mitochondrial bioenergetics) from liver, heart and kidney were evaluated.

Results: No significant differences were found in typical hepatic injury serum markers, showing that AgNPs toxicity is a silent phenomenon. However, our data also demonstrate that the prolonged exposure to a very low dose of AgNPs was sufficient to cause alterations in hepatic mitochondrial function, mainly due to alterations of mitochondrial membrane permeability. Moreover, AgNPs also compromised the electron transfer along the electron transport chain by affecting complex II and IV of the respiratory chain. Mitochondrial function compromised by AgNPs is recovered by pretreatment with NAC, which highlights the crucial role of oxidative stress in AgNPs' toxicity.

Conclusion: Our data show for the first time that even a very low dose of AgNPs can cause harmful effects on mitochondrial function, thus compromising the normal function of the organ, especially the liver, which is the most affected organ of the ones tested.

P034-F | The interplay between cAMP and Sirtuins as a nexus of mitochondrial and metabolic control

J. Amorim*,†,‡; J. Teodoro*,§; R. Silva*; A. Rolo*,§; C. Steegborn¶; D. Sinclair‡; C. Palmeira*,§

*CNC.IBILI – Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal; †IIIUC – Institute of Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; ‡Glenn Labs for the Biological Mechanisms of Aging, Department of Genetics, Harvard Medical School, Boston, USA; §Department of Life Sciences, University of Coimbra, Coimbra, Portugal; ¶Department of Biochemistry, University of Bayreuth, Bayreuth, Germany

Metabolic homeostasis declines with age. Mitochondria are dynamic organelles with a central role in energy metabolism, as well as calcium homeostasis, redox state, differentiation and signaling, and overall cell viability. Consequently, mitochondrial dysfunction in human pathologies such as cancer, inflammation, age related, and metabolic diseases make mitochondrial signaling systems attractive candidates for treatment of these diseases. Sirtuins, of which there are seven (SIRT1-7), are lysine deacylases that play a central role in mitochondrial function, metabolic regulation, and aging. Indeed, efficient mitochondria and adaptive changes in oxidative metabolism were shown to be coordinated by signaling pathways, activated by physiological stimuli, controlling an intense cross talk between nucleus and mitochondrial genomes.

In this study we are using different cell lines, SIRT3$^{-/-}$ mouse embryonic fibroblasts (MEFs), SIRT1$^{-/-}$ MEFs, as well as wild-type MEFs as a control cells. Cells were incubated with specific activators and inhibitors controlling these signaling pathways and mitochondrial function was analyzed.

As a result, we observed an improvement of mitochondrial function in SIRT3$^{-/-}$ MEFs and SIRT1$^{-/-}$ MEFs by observing a dose- and time-dependent increase in mitochondrial membrane potential. Moreover, no changes were observed for reactive oxidative species formation and an increase in nuclear-encoded mitochondrial genes were observed.

In our study, from the molecular to the physiological level, we are starting to understand the crosstalk between two major signaling systems in two cell compartments. Pharmacological or alternative therapies offer promising approaches to target and manipulate these systems leading to the maintenance of mitochondrial content and function, and ultimately prevention of cellular dysfunction. Here, our major goal is to contribute for a better understand on aging and disease processes and provide also opportunities for pharmacological intervention to treat age related diseases.

CARDIOLOGY-CIBERCV

P073-T | Glomerular filtration rate is associated with free triiodothyronine in euthyroid subjects: comparison between various equations to estimate renal function and creatinine clearance

R. Dullaart; L. van Tienhoven-Wind; E. Gruppen; R. Gansevoort; S. Bakker

University of Groningen, Groningen, Netherlands

Background: Effects of variations in thyroid function within the euthyroid range on renal function are unclear. Cystatin C-based equations to estimate glomerular filtration rate (GFR) are currently advocated for mortality and renal risk prediction. However, the applicability of cystatin C-based equations is discouraged in patients with overt thyroid dysfunction, since serum cystatin C and creatinine levels are oppositely affected by thyroid dysfunction. Here, we compared relationships of thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) with various measures of kidney function in euthyroid subjects.

Materials and methods: Relationships of eGFR, based on creatinine (eGFRcrea), cystatin C (eGFRcysC), creatinine + cystatin C combined (eGFRcrea-cysC) and creatinine clearance (CrCl) with TSH, FT4 and FT3 were determined in 2180 euthyroid subjects (TSH, FT4 and FT3 all within the reference range; anti-thyroid peroxidase autoantibodies negative) who did not use thyroid hormones, anti-thyroid drugs, amiodarone or lithium carbonate.

Results: In multivariable models including TSH, FT3 and FT4 together, eGFRcrea, eGFRcysC and eGFRcrea-cysC and CrCl were all positively related to FT3 ($P \leq 0{\cdot}001$), translating into a 2·61 to 2·83 mL/min/1·73 m2 increase in eGFR measures and a 3·92 mL/min increase in CrCl per 1 pM increment in FT3. These relationships with FT3 remained taking account of relevant covariates.

Conclusions: In euthyroid subjects renal function is associated with thyroid function status, especially by serum FT3, irrespective of the eGFR equation applied. In the euthyroid state, cystatin C-based eGFR equations are appropriate to assess the relationship of renal function with variation in thyroid function status.

P074-T | Pre-surgery C-reactive protein levels can predict diabetes remission following bariatric surgery

A. Bonaventura*; L. Liberale*,†; F. Carbone*; A. Vecchié*; R. Cordera‡; G. Adami*; F. Dallegri*,§; F. Montecucco*,§,¶

**Department of Internal Medicine, University of Genoa, Genoa, Italy; †Center for Molecular Cardiology, University of Zürich, Schlieren, Switzerland; ‡Diabetology Unit, Department of Internal Medicine, University of Genoa, Genoa, Italy; §Ospedale Policlinico San Martino, Genoa, Italy; ¶Centre of Excellence for Biomedical Research (CEBR), University of Genoa, Genoa, Italy*

Background: Several studies proved that surgery can improve inflammation parameters, such as C-reactive protein (CRP), especially for metabolic diseases. Few biomarkers have been investigated to potentially predict type 2 diabetes (T2D) remission. We aimed at determining whether pre-surgery serum CRP levels could predict T2D remission in a 3-year follow-up period among patients undergoing bariatric surgery, especially biliopancreatic diversion (BPD).

Materials and methods: This study has been conducted from July 2007 to July 2009 at the Surgical Department of the University of Genoa, Italy. Forty-four patients with T2D undergoing BPD ($n = 38$) or Roux-en-Y gastric bypass (RYGB) ($n = 6$) have been included. After a multidisciplinary evaluation, including a diabetologist, a dietitian, and a nurse, patients have been addressed to BPD or RYGB. The primary endpoint was to evaluate whether pre-surgery CRP levels could predict T2D partial remission (according to the American Diabetes Association definition) at 3-year follow-up. Secondary endpoints were to assess whether glycemic, lipid, and inflammatory parameters changed during the follow-up period.

Results: At baseline, patients with T2D ranged from overweight to morbid obesity and presented with mild dyslipidemia and a low-grade inflammation. Bariatric surgery improved body weight, lipid and glycemic profile both at 1- and 3-year follow-up. Pre-surgery CRP levels progressively decreased already after the first month and also at 1- and 3-year follow-up. Among inflammatory pre-surgery biomarkers, only high CRP levels have been demonstrated to predict partial T2D remission after 3 years. Multivariate analysis confirmed the predictive value of pre-surgery CRP independently of age, gender, type of surgery, and body mass index.

Conclusions: Bariatric surgery, in particular BPD, improved both metabolic and inflammatory biomarkers at 1- and 3-year follow-up. Pre-surgery high CRP levels predicted 3-year partial T2D remission, thus identifying a promising target population who can benefit from bariatric surgery, especially BPD.

P075-T | Proteomic signature of circulating extracellular vesicles in dilated cardiomyopathy

S. Roura*,†; A. Gámez-Valero‡; J. Lupón†,§; C. Gálvez-Montón*,†; F.E. Borràs‡; A. Bayes-Genis*,†,§

*ICREC Research Programme, Fundació Institut d'Investigació Germans Trias i Pujol (IGTP), Badalona, Spain; †CIBERCV, Instituto de Salud Carlos III, Madrid, Spain; ‡REMAR-IVECAT Group, IGTP, Badalona, Spain; §Cardiology Service, Germans Trias i Pujol University Hospital, Badalona, Spain

Background: Dilated cardiomyopathy (DCM) remains a major cause of heart failure and carries a poor prognosis despite important advances in recent years. Better disease characterization using novel molecular techniques is needed to refine its progression. This study explored the proteomic signature of plasma-derived extracellular vesicles (EVs) obtained from DCM patients and healthy controls using size-exclusion chromatography (SEC).

Material and methods: Purified SEC-EV fractions were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). Raw data obtained from LC-MS/MS were analyzed against the Uniprot human database using MaxQuant software. Additional analyses using Perseus software were based on the Intensity-Based Absolute Quantification (iBAQ) values from MaxQuant analyses.

Results: A total of 90·07 ± 21 proteins were identified (183 different proteins) in the DCM group and 96·52 ± 17·91 proteins (227 different proteins) in the control group. A total of 176 proteins (74·6%) were shared by controls and DCM patients, whereas 51 proteins were exclusive for the DCM group and 7 proteins were exclusive for the control group. Fibrinogen (alpha, β, and γ chain), serotransferrin, alpha-1-antitrypsin, and a variety of apolipoprotein family members (C-I, C-III, D, H or β-2-glycoprotein, and J or clusterin) were clustered in SEC-EVs derived from DCM patients relative to controls ($P < 0·05$). Regarding Gene Ontology analysis, response to stress and protein activation-related proteins were enriched in DCM-EVs compared to controls.

Conclusions: Overall, our results show the distinct proteomic signature of circulating EVs from DCM patients compared to those from healthy subjects. We also reveal that SEC obtains highly-purified EV fractions from peripheral blood samples for subsequent use in determining disease-specific proteomic signatures.

P076-T | Metabolic profiling reveals significant alterations in glycerophospholipid and sphingolipid content in hearts from rats treated with relaxin

A. Aragón-Herrera*; S. Feijóo-Bandín*,†; V. Abella*,†; O. Gualillo‡; E. Roselló-Lletí†,§; M. Portolés†,§; M. Rivera†,§; M. Bigazzi¶; D. Bani¶; J.R. González-Juanatey*,†; F. Lago*,†

*Cellular and Molecular Cardiology Unit and Department of Cardiology, Institute of Biomedical Research (IDIS-SERGAS), Santiago de Compostela, Spain; †CIBERCV, Institute of Health Carlos III, Madrid, Spain; ‡Neuroendocrine Interaccions in Rheumatology and Inflammatory Diseases Unit, Institute of Biomedical Research (IDIS-SERGAS), Santiago de Compostela, Spain; §Cardiocirculatory Unit, Health Institute of La Fe University Hospital (IIS La Fe), Valencia, Spain; ¶Department of Experimental and Clinical Medicine, Research Unit of Histology & Embryology, University of Florence, Florence, Italy

Background: Serelaxin, the recombinant form of human relaxin-2, is under clinical trials with patients with acute heart failure to elucidate if it is capable to improve mortality and to ameliorate the symptoms.

Nowadays, the possible effects of the hormone relaxin on cardiac metabolism have been unexplored. Our objective is to study its in vivo effects on the cardiac metabolic profile.

Material and methods: Male Sprague-Dawley rats ($n = 10$ per group) were treated with human recombinant relaxin-2 using subcutaneous osmotic minipumps filled with human recombinant relaxin-2 solution (0·4 mg/kg/d) or vehicle. The plasma levels of relaxin-2 achieved after the treatment were measured using ELISA. The metabolic profiling of atrial tissues was analysed using four UHPLC-MS based platforms: 1-fatty acyls, bile acids, steroids and lysoglycerophospholipids, 2-glycerolipids, glycerophospholipids, sterol lipids and sphingolipids, 3-aminoacids and 4-polar metabolites profiling, including Central Carbon Metabolism.

Results: The atrial tissues obtained from relaxin-2 treated rats showed a significant increase in different types of sphingolipids and glycerophospholipids compared to controls. In the sphingolipids, there are differences in two ceramides (Cer): Cer(d18:1/23:0) ($P = 0·04$) and Cer(d43:1) ($P = 0·02$) and in five sphingomyelins (SM): SM(42:1) ($P = 0·03$), SM(d18:0/16:0) ($P = 0·005$), SM(d18:0/22:0) ($P = 0·04$), SM(d18:1/16:0) ($P = 0·04$) and SM(38:1) ($P = 0·03$). In the glycerophospholipids, there are differences in three phosphatidylethanolamines (PE): PE(18:2/18:2) ($P = 0·04$), PE(16:0/22:6) ($P = 0·02$) and PE(18:0/22:6) ($P = 0·00005$) and in twelve phosphatidylcholines (PC): PC(15:0/20:4) ($P = 0·04$), PC(16:0/20:4) ($P = 0·01$), PC(15:0/22:6) ($P = 0·04$), PC(16:0/22:6) ($P = 0·001$), PC(18:0/22:4) ($P = 0·01$), PC(18:0/22:6) ($P = 0·002$), PC(18:1/22:6) ($P = 0·0001$), PC(40:5) ($P = 0·01$), PC(40:8) ($P = 0·002$), PC(40:1) ($P = 0·009$), PC(38:5) ($P = 0·003$) and PC(O-38:4) ($P = 0·04$).

Conclusions: Relaxin is able to induce significant changes in the lipidome of rat cardiac tissue; increasing the levels of Cer and SM and affecting the PC/PE ratios. All this molecules are highly bioactive compounds with profound effects on cell regulation and with a master regulatory role in the physiology and the physiopathology of the heart.

P077-T | Leukotriene B4 plasma levels are associated to AAA prevalence and progression

R. Roldan-montero*,†; J.S. Lindholt‡; D. Martinez-Lopez*; L.M. Blanco-Colio*,†; J.L. Martin-Ventura*,†

**Fiis-fundacion Jimenez Diaz, Madrid, Spain; †CIBER-CV; ‡Elitary Research Centre of Individualized Medicine in Arterial Disease (CIMA), Department of Cardiothoracic and Vascular Surgery, Odense University Hospital, Odense, Denmark*

Background: Previous human and experimental studies have suggested a role of arachidonic acid pathway in abdominal aortic aneurysm (AAA). Arachidonic acid-derived metabolites, such as leukotriene B4 (LTB4), has been previously observed in human AAA tissue, potentially contributing to neutrophil recruitment. Our aim was to investigate the potential association of LTB4 plasma levels with AAA presence and progression.

Material and methods: We obtained blood samples from 493 AAA patients (maximal aortic diameter ≥30 mm) within a population-based ultrasound-screening trial in Danish men and from 198 age-matched screened negative controls. LTB4 plasma levels were assessed by ELISA. During a median follow-up of around 5 years, 141 AAA patients reached criteria for vascular surgical repair.

Results: LTB4 plasma levels in AAA patients were higher than in controls (976·6 [432·9–16 983·7] vs 316·5 [44·9–613·5] pg/mL, $P < 0{\cdot}001$), and individuals in the upper tertile of LTB4 at baseline had higher probability of having AAA (odds ratio = 8·3, 95% confidence interval, 4·2; 16·5, $P < 0{\cdot}001$). AAA patients at the upper tertile of LTB4 at baseline had a 60% higher risk of needing surgical repair during the follow-up (hazard ratio = 1·6, 95% confidence interval, 1·2; 2·3, $P = 0{\cdot}003$).

Conclusions: LTB4 is associated to AAA presence and progression suggesting its potential use as a prognostic marker. Future studies are needed to clarify the pathogenic role of LTB4 in AAA.

P078-T | Coronary artery disease with silent myocardial ischemia

A. Abdrahmanova*; I. Abdulyanov†; R. Gaifullina*; L. Valeeva*

**Kazan Federal University, Kazan, Russian Federation; †Interregional Clinical Diagnostic Center, Kazan, Russian Federation*

Background: The aim of the research is analysis of recent publications devoted to the features of coronary lesions in patients with painless myocardial ischemia from coronary angiography and comparison of literature data with own data.

Materials and methods: Retrospectively, the data of selective coronary angiography of 84 patients, which were carried out during the period from January 2011 to December 2015, in the conditions of the Interregional Clinical Diagnostic Center.

Results and discussion: Out of 84 patients 7 (8·3%) had malaisienne coronary arteries, 16 (19%) – lose 1 vessel, 24 (28·6%) – lose 2 vessels, 37 (47·4%) – multiple lesions of coronary arteries. The most commonly affected was the anterior interventricular artery (in 69 patients [89·6%] is P 0·005). The analysis of the level of the lesion demonstrated that it is dominated by middle and proximal segments of the anterior interventricular artery (44·5% and 36·6%), right coronary artery (40·8% and 20%), circumflex artery (34·4–40·9%). Hemodynamically significant stenoses were found in the anterior interventricular artery (33%), right coronary artery (24%), circumflex artery (41·3%), left main coronary artery (63·7%). Most often, marveling at the coronary artery of the second order was a branch of a blunt edge (32·5%), hemodynamically significant stenosis was found in 48·6% of cases.

Conclusions: Conducted research confirms the point of view of multi-vessel lesion of coronary arteries in these patients, with a predominance of lesions of the anterior interventricular artery with a high level of critical stenosis. The inconsistency of the available data on severity of prevalence of coronary artery in patients with silent ischemia according to current literature requires further research. Work supported by Program of Competitive Growth of Kazan Federal University. The study was funded by the Russian Foundation for Basic Research (grant No. 16-06-01064).

P079-T | Surgical treatment of patients with aortic stenosis and low ejection fraction

A. Abdrahmanova*; I. Abdulyanov†; R. Gaifullina*; R. Khasanova*; Y. Oslopova*; F. Rizvanova‡

*Kazan Federal University, Kazan, Russian Federation; †Interregional Clinical Diagnostic Center, Kazan, Russian Federation; ‡Kazan State Medical University, Kazan, Russian Federation

Background: The aim of the study is to analyse modern mortality in case of isolated AS replacement from 1% to 4·8%.

Materials and methods: The study included 38 patients (LVEF ≤ 40%) and the maximum transvalvular gradient of 40 mmHg. A high risk of surgery was expected, according to Euroscore II, nearly 4–5%, the average score was 5·8 ± 2·4.

Results: After surgery LVEF increased from 36·3 ± 4·1% to 39·7 ± 6·3% ($P = 0·003$). Improvement of the LVEF occurred in 26 (68%) patients in the nearest time. In 32% of cases (12 patients), LVEF either remained unchanged or decreased. Mean transvalvular gradient on the artificial valve was 27·3 ± 10·7 mmHg, which resulted in an increase in CO and CI ($r \leq 0·05$), but 4 (10%, 5%) patients had a low CI (less than 2 L/min/m^2). Also, there was a decrease in the thickness of the IVS by 15% on average (from 1·35 ± 0·2 to 1·18 ± 0·1 cm [$r \leq 0·01$]). The elimination of the AS led to a decrease in pressure in the pulmonary circulation, and consequently to a decrease in pulmonary artery pressure (from 54·4 ± 16·1 to 35·7 ± 7 mmHg [$r \leq 0·01$]). CO (from 3·9 ± 1·6 to 4·6 ± 0·9 L/min [$r \leq 0·002$]) and CI (from 2·14 ± 0·6 to 2·5 ± 0·6 L/min/m^2 [$r \leq 0·001$]) in the postoperative period in the group of patients reached standard values on average. Reduced pressure in the LV and the load on the LV wall quickly affected the thickness of the myocardium, with further reduction of LV hypertrophy (IVS size decreased). EDD decreased more significantly from 5·7 ± 0·8 to 5·2 ± 0·6 cm ($P = 0·04$).

Conclusion: Surgical treatment of patients with severe aortic stenosis with low left ventricular ejection fraction has shown good results in contrast to the proposed stratification of the surgery risk. Work supported by Program of Competitive Growth of Kazan Federal University.

P080-T | Hystochrome for acute coronary syndrome patients with cardiac rhythm disorder

R. Gaifullina*; Z. Kim†; L. Valeeva*

*Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation

Background: The aim of the study is estimation of clinical and antiarrhythmic effectiveness of hystochrome for acute coronary syndrome patients with ST segment depression depicted on electrocardiography during anginose stroke.

Materials and methods: We have investigated 59 patients with acute coronary syndrome whose anginose stroke was accompanied by horizontal or oblique decrease of ST segment to 1–7 mm after 0·08 seconds after J with 50–120 beats/min heart rate. Acute coronary syndrome of 31 patients (52·54%) transformed to acute myocardial infarction – 11 with Q wave and 20 without Q wave. In anamneses of 26 people (44·06%) there was previously carried myocardial infarction; repeated myocardial infarction developed at 11 patients (18·64%). 21 people (47·45%) were diagnosed with effort angina III-IV functional class which lead to acute coronary syndrome. ECC 25 patients (42·37%) demonstrated rhythm disorders, including supraventricular extrasystole – 4 people (6·78%), ventricular arrhythmia of 1st gradation – 3 people (5·08%), 2nd gradation 8 people (13·56%), permanent form of atrial fibrillation – 6 people (10·16%) and paroxysmal form of atrial fibrillation 4 people (6·78%). The following criteria were used to find out VLP: length of QRS complex >114 ms; signal of the terminal part of QRS complex <40 mkV; quadratic mean of tension in last 40 ms of QRS complex <25 mkV.

Results: In the course of the treatment by hystochrome rhythm disturbances were not registered. Also, decrease of general duration of ischemic attacks (to 59%), ΣST valuable (to 67·6%) and decrease of nitro-glycerine quantity (to 62·2%) were registered.

Conclusion: The research has proved cardioprotective and antiarrhythmic effectiveness of hystochrome for acute coronary syndrome patients with ST segment depression depicted on ECG. Work supported by Program of Competitive Growth of Kazan Federal University. The study was supported by the Russian Foundation for Basic Research (16-06-0106)

P081-T | Contribution of chronic endoplasmic reticulum stress and oxysterols to abdominal aortic aneurysm disease

M. Galán*,†; M. Navas-Madroñal*; C. Rodriguez‡,†; J. Fité*; L. Vila*,†; J. Martínez-González†,§; M. Camacho*,†

*Institut de Recerca del Hospital de la Santa Creu i Sant Pau, Instituto de Investigación Biomédica Sant Pau (IIB Sant Pau), Barcelona, Spain; †CIBER de Enfermedades Cardiovasculares, Madrid, Spain; ‡Institut de Recerca del Hospital de la Santa Creu i Sant Pau-Programa ICCC, IIB Sant Pau, Barcelona, Spain; §Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), IIB Sant Pau, Barcelona, Spain

Background: Abdominal aortic aneurysm (AAA) is a degenerative vascular disease with high morbidity and mortality. It has a prevalence of 4–8% in men over the age of 65 years that is increasing in association with the aging population. It is thought to be caused by a combination of multiple risks factors including smoking, aging, hypertension and chronic inflammation. However, its etiology is complex and remains to be elucidated. We aim to determine the role of endoplasmic reticulum (ER) stress in AAA and whether the profile of oxysterols such as 7-ketocholesterol (7-KC), an ER stress inducer in vascular cells, is altered in plasma of patients.

Materials and methods: The ER stress activation was studied by real time PCR, Western Blot and immunostaining in a cohort of AAA samples from 100 patients compared with abdominal aorta from 20 healthy donors. The quantification of oxysterols was performed by liquid chromatography-(APCI)-mass spectrometry (LC-[APCI]-MS/MS) in plasma.

Results: ER stress activation was assessed by enhanced gene expression of ATF6, IRE-1, XBP-1 and CHOP and by an increase in protein levels of active ATF6, active XBP1 and the pro-apoptotic protein CHOP in AAA. This was accompanied by an exacerbated apoptosis in the vascular wall of AAA (measured by TUNEL and cleaved caspase-3) and by an increase in NADPH oxidase subunits expression and ROS production (studied by dihydroethidium and Mitosox red staining). Plasma levels of 7-KC were significantly augmented in AAA patients while the levels of hydroxy cholesterols (7α-HC, 24-HC and 27-HC) were significantly lower in patients compared to healthy donors.

Conclusion: Our results evidence that ER stress is involved in the pathophysiology of aneurysm and suggest that the alteration in circulating levels of oxysterols could contribute to the development of AAA.

This work was funded by the ISCIII under the program Miguel Servet I (CP15/00126) and FEDER.

P082-T | Inhibition of enzymes involved in collagen cross-linking reduces vascular smooth muscle cell calcification

C. Rodríguez*,†; E. Jover‡; A. Silvente‡; F. Marín†,‡; J. Martínez-González†,§; M. Orriols†; C.M. Martínez¶; C.M. Puche‡; M. Valdés†,‡; D. Hernández-Romero†,‡

*Institut de Recerca del Hospital de la Santa Creu i Sant Pau-Programa ICCC, IIB-Sant Pau, Barcelona, Spain; †CIBER de Enfermedades Cardiovasculares (CIBERCV), Spain; ‡Hospital Clínico Universitario Virgen de la Arrixaca, Universidad de Murcia, IMIB-Arrixaca, Murcia, Spain; §Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), IIB-Sant Pau, Barcelona, Spain; ¶CIBER de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain

Background and objectives: Vascular smooth muscle cells (VSMC) transdifferentiate into osteoblast-like cells during vascular calcification inducing an active remodelling and calcification of the extracellular matrix (ECM). Intracellular and extracellular enzymes, such as lysyl hydroxylase 1 (PLOD1) and lysyl oxidase (LOX), contribute to ECM maturation and stabilization. We aimed to assess the contribution of these enzymes to hyperphosphatemia (HPM)-induced calcification.

Material and methods: Studies were performed in human and murine VSMC (hVSMC, mVSMC). Aortic rings for ex vivo calcification studies and mVSMC were isolated from both wild-type and transgenic mice over-expressing LOX in VSMC (TgLOX). LOX, PLOD and osteoblast marker expression was analyzed by real-time PCR and western-blot. Calcium content was determined by the o-cresolphthalein method and visualized by von Kossa and Alizarin Red staining. Immunohistochemical analysis were performed in femoral or popliteal human atherosclerotic lesions. ECM collagen I structure was analyzed by confocal microscopy.

Results: hVSMC and mVSMC were differentiated into functional osteoblast-like cells by HPM conditioning. HPM promoted ECM calcification and up-regulated osteoblast markers associated to an induction of LOX and PLOD1 expression. mVSMC from transgenic mice over-expressing LOX (TgLOX) exhibited an increase in HPM-dependent calcification and osteoblast commitment compared with wild-type cells. Similarly, enhanced HPM-induced calcification was detected in aorta from TgLOX. Conversely, both β-aminopropionitrile (BAPN; a LOX inhibitor), and LOX knockdown abrogated VSMC calcification and transdifferentiation. Interestingly, we found a significant positive association between LOX expression and vascular calcification in human atherosclerotic lesions. Likewise, 2,2′-dipyridil (an inhibitor of PLOD) and PLOD1 knockdown impaired HPM-induced ECM mineralization and osteoblast commitment.

Conclusions: Our findings identify LOX and PLOD as critical players in vascular calcification and highlight the importance of ECM remodelling in this process.

P083-T | NOR-1 overexpression potentiates cardiac hypertrophy: results from a new transgenic animal model

L. Cañes*,†; I. Martí-Pàmies†; J. Alonso†; M. Galan‡; P. Muniesa§; J. Osada§,¶; C. Rodríguez†,‡; J. Martínez-González*,†

**Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), IIB-Sant Pau, Barcelona, Spain; †CIBER de Enfermedades de Cardiovasculares (CIBERCV), Madrid, Spain; ‡Institut de Recerca del Hospital de la Santa Creu i Sant Pau-Programa ICCC, IIB-Sant Pau, Barcelona, Spain; §Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain; ¶CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Madrid, Spain*

Background and objective: NOR-1 is a transcription factor belonging to the NR4A subfamily of orphan nuclear receptors. Our group has shown the key role of NOR-1 in the activation of vascular smooth muscle cells and vascular remodelling. Recently a role for NOR-1 in cardiac hypertrophy has been suggested. Our objective was to assess the impact of NOR-1 overexpression on ventricular function and cardiac hypertrophy in a transgenic mouse model (TgNOR1) with a strong cardiac expression of human NOR-1.

Material and methods: Transgenic mice carrying the human NOR-1 cDNA under the control of the CAG promoter were generated. These animals exhibited high overexpression of human NOR-1 in the heart. Cardiac hypertrophy was induced in transgenic and control animals (WT) by subcutaneous infusion of angiotensin II (AngII; 1000 ng/kg/min, 4 weeks) or Isoprenaline (ISO; 15 mg/kg/day, 1 week) using osmotic mini-pumps. Cardiac function was monitored by ultrasounds and systolic blood pressure were measured weekly. Cardiac expression of hypertrophic markers was analysed by RT-PCR. The cardiomyocyte cross-sectional area and fibrosis were evaluated by hematoxylin-eosin and picrosirius red staining, respectively.

Results: AngII and ISO induced a similar increase in systolic blood pressure in both TgNOR1 and WT animals. NOR-1 transgenesis, however, aggravated AngII- and ISO-induced cardiac hypertrophy (LVmass: WT-AngII 114·6 ± 5·6 vs TG-AngII 134·9 ± 6·9, $P < 0·05$; WT-ISO 137·1 ± 5·7 vs TG-ISO 157 ± 4·2; $P < 0·05$). In accordance, NOR-1 over-expression increased the cardiomyocyte cross-sectional area and fibrosis, and exacerbated the expression of cardiac hypertrophy (Bnp1, Acta1, Myh7) and fibrotic markers (Col1a1 and Col3a1). Furthermore, significant differences were found in other parameters such as ejection fraction and LV anterior wall thickness.

Conclusions: Our findings indicate that NOR-1 play a role in cardiac hypertrophy. We have generated a new animal model useful to assess cardiac hypertrophy pathophysiology and to address pharmacological preclinical studies.

Funded by MINECO-ISCIII: SAF2015-64767R and PI15/01016. Co-funded by FEDER.

P084-T | TMAO new biomarker for disease severity in peripheral arterial disease

C. Roncal*,†,‡; E. Martinez-Aguilar‡,§; J. Orbe*,†,‡; A.E.-H. de Mendoza¶; J.A. Rodriguez*,†,‡; S. Fernandez-Alonso‡,§; L. Fernandez-Alonso‡,§; J. Oyarzabal¶; J.A. Paramo*,†,‡,**

**Laboratory of Atherothrombosis, Program of Cardiovascular Diseases, CIMA-Universidad de Navarra, Pamplona, Spain; †CIBER-CV, Spain; ‡IdiSNA, Spain; §Departamento de Angiología y Cirugía Vascular, Complejo Hospitalario de Navarra, Pamplona, Spain; ¶Small Molecules Platform, Program of Molecular Therapies, CIMA-University of Navarra, Pamplona, Spain; **Hematology Service, Clínica Universidad de Navarra, University of Navarra, Pamplona, Spain*

Background: Intestinal microbiome is essential for the generation of trimethylamine (TMA), which is converted to trimethylamine-N-Oxide (TMAO) in the liver. TMAO levels have been associated to atherosclerosis development and elevated cardiovascular (CV) risk in preclinical and clinical studies. The aim of this study was to determine the diagnostic and prognostic value of TMAO in peripheral arterial disease (PAD).

Material and methods: 265 symptomatic PAD patients were included in the study (mean age 70 years, 80% men). CV risk factors and pharmacological treatments were recorded at recruitment. All-cause and CV mortality rates were 33% and 14% respectively over a mean follow-up of 41 ± 27 months. TMAO levels were measured in plasma by mass spectrometry.

Results: TMAO levels were inversely associated to the uncle brachial index (ABI, $r = -0·235$, $P = 0·001$) and the glomerular filtration rate (GFR, $r = -0·310$, $P < 0·001$), and were increased in critical limb ischemia (CLI, $n = 116$) compared to intermittent claudication (IC, $n = 149$) (μmol/L: 2·2 ± 3·0 IC vs 5·8 ± 7·5, $P < 0·001$). Likewise, elevated levels of TMAO (Tertile 3, T3) were independently associated to disease severity (T3 OR: 11·2; 95% CI: 3·2–39·5). In the follow up, patients in the higher tertiles of TMAO presented increased frequency of both CV (T1: 2%, T2: 14% and T3: 21%, $P < 0·001$), and all cause death (T1: 19%, T2: 36% and T3: 44%, $P < 0·001$). When adjusted by other confounding factors, the association between TMAO and mortality, either CV (T3 HR: 2·0;

95% CI: 0·4–11·6) or global (T3 HR: 0·7; 95% CI: 0·32–1·6), was no longer significant if renal disease was included in the analysis.

Conclusions: TMAO levels might represent a new diagnostic biomarker in PAD. The lack of association between outcome and TMAO after multifactorial analysis might be related to the high percentage of patients with coexisting renal disease, which is known to reduce TMAO clearance and therefore facilitate its accumulation in blood.

P085-T | PGE_2 derived from microsomal prostaglandin e synthase-1 induces vascular oxidative stress from nadph oxidase and mitochondria and mediates vascular dysfunction in hypertension

A.B. Garcia-Redondo*,†; M.S. Avendaño*; A. Aguado*; M. González-Amor*; S. Martínez-Revelles*,†; M. Camacho†,‡; V. Cachofeiro†,‡; M.J. Alonso†,¶; G. Zalba†,**; M. Salaices*,†; A. Briones*,†

*Departmento de Farmacología, Universidad Autónoma de Madrid, Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain; †Ciber de Enfermedades Cardiovasculares, Spain; ‡Laboratorio de Angiología, Biología Vascular e Inflamación.Instituto de Investigación Biomédica (IIB Sant Pau), Barcelona, Spain; ¶Departamento de Ciencias Básicas de la Salud, Universidad Rey Juan Carlos, Madrid, Spain; **Departmento de Bioquimica y Genetica, Universidad de Navarra y IdiSNA, Instituto de Investigacion Sanitaria de Navarra (IdiSNA), Pamplona, Spain*

Background: Cyclooxygenase-derived products contribute to Angiotensin II (AngII)-induced vascular dysfunction. Moreover, microsomal prostaglandin E synthase-1 (mPGES-1), the downstream enzyme responsible for prostaglandin E_2 (PGE_2) synthesis in inflammatory conditions, is increased in hypertensive vessels. We evaluated the role of mPGES-1-derived PGE_2 in the vascular dysfunction and remodeling in AngII-induced hypertension and the contribution of oxidative stress to these effects.

Material and methods: We used arteries from untreated and AngII-infused mPGES-$1^{-/-}$ and mPGES-$1^{+/+}$ mice, vascular smooth muscle cells (VSMC) exposed to PGE_2 and phagocytic cells from normotensive and hypertensive patients. mPGES-1 deletion decreased the augmented wall: lumen ratio, vascular stiffness and collagen deposition and normalized the altered elastin structure observed in mesenteric arteries from hypertensive animals.

Results: In AngII-infused mice, mPGES-1 deletion also prevented: (i) the increased gene expression of connective tissue growth factor, plasminogen activator inhibitor-1, tumor necrosis factor α and the macrophage marker Mac-3; (ii) the increased vasoconstrictor responses and the endothelial dysfunction; (iii) the increased NADPH Oxidase activity and mitochondrial dysfunction and (iv) the increased reactive oxygen species production and the reduced NO bioavailability. In VSMC and/or aortic segments PGE_2 increased NADPH Oxidase expression and activity and reduced mitochondrial membrane potential, effects that were abolished by EP_1 and EP_3 receptor antagonists and JNK and ERK1/2 inhibition. In human phagocytic cells, there is a positive correlation between NADPH Oxidase activity, mPGES-1 gene expression and hypertension.

Conclusion: mPGES-1-derived PGE_2, via EP_1/EP_3/JNK/ERK1/2, is involved in the vascular remodeling and stiffness and endothelial dysfunction in AngII-induced hypertension, by an increase of oxidative stress produced by NADPH Oxidase and mitochondria.

P086-T | Human lysyl oxidase overexpression does not modify infarct size in mice

L. Valls-Lacalle*,†; M. Pecoraro*,†; S. Varona†,‡; J. Martínez-González†,‡; C. Rodríguez†,§; A. Rodríguez-Sinovas*,†; D. García-Dorado*,†

Vall D'hebron University Hospital and Research Institute, Cardiovascular Diseases Research Group, Barcelona, Spain; †CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; ‡Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), IIB-Sant Pau, Barcelona, Spain; §Institut de Recerca del Hospital de la Santa Creu i Sant Pau-Programa ICCC, IIB-Sant Pau, Barcelona, Spain

Background: Lysyl oxidase (LOX) is an extracellular enzyme produced by fibroblasts that plays a critical role in collagen maturation. LOX oxidizes peptidyl lysines to semi-aldehydes, which then condense with neighbouring amino or peptidyl groups creating intra- and inter-molecular covalent cross-links. Previous studies have demonstrated that LOX overexpression in mice accelerates and aggravates angiotensin II-induced cardiac remodeling, and is associated with cardiac detrimental effects in rats with aortocaval fistula-induced volume overload. However, the effects of LOX overexpression after myocardial infarction have not been previously analyzed.

Material and methods: To assess whether LOX overexpression can modify acute ischemia-reperfusion injury, hearts were isolated from wild-type or TgLOX animals, which overexpress human LOX, and submitted to 35 minutes of global ischemia followed by reperfusion (n = 5/group). Left ventricular developed pressure and lactate dehydrogenase release (LDH) were continuously monitored, and infarct size was determined by 2,3,5-triphenyltetrazolium chloride (TTC) staining at the end of the experiments. To investigate whether LOX overexpression

has any influence on postinfarct cardiac remodeling, additional experiments (n = 6–7/group) were performed in an vivo mice model of left anterior descending coronary artery occlusion (45 minutes) followed by 28 days of reperfusion. Left ventricular dimensions and function were assessed by serial echocardiography, and infarct size was determined by picrosirius red staining.

Results: Acute ischemia-reperfusion injury was not modified by human LOX overexpression in isolated mice hearts (infarct size: 56·24 ± 9·44% and 48·63 ± 2·99% of cardiac weight in wild-type and TgLOX hearts, respectively; cumulative LDH: 132·19 ± 37·58 and 134·35 ± 14·24 U/g dry tissue/15 minutes). Similarly, human LOX overexpression did not modify changes in cardiac dimensions or function 28 days after myocardial infarction, nor infarct size assessed by picrosirius red (20·29 ± 3·10% vs 21·83 ± 2·83% of left ventricular area).

Conclusions: Human LOX overexpression does not modify infarct size or postinfartion cardiac remodeling following transient coronary occlusion in mice.

P088-T | Importance of NT pro BNP in management of chronic ischemic patient to prevent heart failure

I. Dimitriu; D. Voiculescu; A. Lacraru; C. Sinescu

University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

Background: To prove the importance of NTproBNP for preventing heart failure at ischemic patients.

Materials and methods: We included 196 patients who were presented at hospital during 2 years, with myocardial infarction, angioplasty or aortocoronary bypass in their medical history. We excluded patients who are already diagnosed with heart failure or who had current symptoms of heart failure. We randomized patients into 2 equal groups: a control group and an intervention group. NTproBNP value was determined for all patients. In the intervention group patients were treated according to the NTproBNP. Patients with NTproBNP < 125 pg/dL (54 patients) received standard treatment for their symptoms. Patients with NTproBNP > 125 pg/dL (44 patients) were the ones on which we intervened to prevent heart failure. They were investigated by cardiac ultrasound and other tests and they received specific treatment. Patients in the control group received standard treatment regardless of the NTproBNP value.

Results: After 2 years, the end points were: diagnosis of heart failure left ventricular dysfunction, death from any cause, the rate of hospitalizations for cardiovascular pathology. After 2 years in the control group were twenty-one (21·8%) patients who developed heart failure compared to fourteen (14·2%) patients in the intervention group. Fifty four (59·1%) patients were diagnosed with left ventricular systolic dysfunction, compared to thirty-nine (39·7%) in the intervention group. Also, and rate of admissions for cardiovascular pathology was higher in the control group thirty-two (22·4%) vs nine (9, 18%) in the intervention group.

Conclusions: Patients in the intervention group, in which the value of NTproBNP was used in choosing therapeutic management, had lower rate of incidence of heart failure or cardiovascular events than patients in the control group. The NTproBNP value in ischemic patients without heart failure can detect patients at risk of developing heart failure. And more, medical intervention guided by NTproBNP can prevent or delay heart failure.

P089-T | Oral pharmacological inhibition of calpains attenuates isoproterenol-induced myocardial hypertrophy and fibrosis

D. Aluja*; J. Inserte*; J. Castellano*; P. Penela†; F. Mayor†; D. Garcia-Dorado*

**Cardiovascular Diseases Research Group, Vall de Hebron Research Institute, CIBER-CV, Barcelona, Spain; †Departamento de Biología Molecular y Centro de Biología Molecular Severo Ochoa, CSIC-UAM, CIBER-CV, Madrid, Spain*

Background: Myocardial remodeling is an important cause of heart failure and a major medical and social problem. Our group has demonstrated that pharmacological inhibition of the Ca^{2+}-dependent proteases calpains attenuates post-infarction remodeling and heart failure. Recent data suggest that calpain activity is elevated in non-ischemic cardiomyopathies and that G-protein coupled receptor kinase 2 (GRK2) promotes cardiac hypertrophy.

Objective: To determine the therapeutic benefit and mechanism of inhibiting calpain activity in pathological myocardial remodeling of non-ischemic cause.

Material and methods: The calpain inhibitor SNJ-1945 was administered orally once a day to male Sprague-Dawley rats or global hemizygous GRK2 mice treated with 5 mg/kg/day isoproterenol (ISO) intraperitoneally for 1 week. Hearts were compared with vehicle-treated and ISO treated rats and mice not receiving the calpain inhibitor.

Results: At 7 days of ISO treatment, calpain-1 was overexpressed and correlated with increased calpain activity, ventricular hypertrophy and interstitial fibrosis. Oral co-administration of SNJ-1945 attenuated calpain activation and reduced heart weight, septal and left ventricular posterior wall thicknesses, cardiomyocyte cross-sectional area,

β-MHC/α-MHC ratio and ANP and BNP mRNA levels. In addition, SNJ-1945 reduced the intraventricular collagen deposition by preventing TGFβ/SMAD-dependent fibroblast activation. Treatment with ISO increased GRK2 protein levels by a mechanism dependent on calpain activation while downregulation of GRK2 expression prevented ISO remodeling independently of calpain activity.

Conclusions: Chronic oral administration of SNJ-1945 attenuates isoproterenol-induced myocardial remodeling by mechanisms involving the modulation of GRK2 protein content. Pharmacological calpain inhibition may be an effective therapeutic strategy to limit adverse ventricular remodeling of non-ischemic origin.

P090-T | Epigenetic regulation through miR-139-5p during human monocyte-to macrophage differentiation

R. Escate*,†; T. Padro*,†; L. Badimon*,†

**Program ICCC-Institut Català de Ciències Cardiovasculars, IR-Hospital De La Santa Creu I Sant Pau, Barcelona, Spain; †CiberCV, Institute Carlos III, Barcelona, Spain, Barcelona, Spain*

Innate immunity cells are key players for the inflammatory processes during atherosclerosis. Monocyte-to-macrophage differentiation is a complex dynamic process resulting in heterogeneous cell populations with different inflammatory patterns. Until now, the epigenetic regulation occurring during monocyte differentiation remains unclear. This study was aimed to identify a differential miRNA-pattern related with monocyte-to-macrophage differentiation, highlighting changes with potential relevance in atherosclerosis progression.

Methods: Studies were made in human blood monocytes (MO) obtained from healthy donors and differentiated (7 days in culture) into macrophages (MAC). miRNA profile was determined using Taqman array microfluidic card. miRNA and gene-transcript levels were assessed by real-time PCR. Target gene prediction was performed by Ingenuity Pathway Analysis (IPA).

Results: Based on the profiler PCR Array analysis, 74 miRNAs were consistently expressed in innate immune cells. Eighteen miRNAs, involved in regulation of inflammatory pathways and cell development, according to the IPA analysis, were differentially expressed in MAC compared to MO (17 upregulated miRNA: 1·5–50-fold; 1 downregulated miRNA: 18-fold). Top-change referred to miR-139-5p (>50-fold increase; $P = 0{\cdot}034$), miRNA associated with cell differentiation. The increase in miR-139-5p levels occurred along with changes in the transcripts of CD68 and CD14, markers of the MAC-phenotype, and changes in gene expression of ANK2, an adapter protein that helps to organize the cell-cytoskeleton and progressively increased during MO-to-MAC differentiation (30-fold higher in MAC vs MO; $P = 0{\cdot}001$). MO transfection with antagomiR-139-5p induces upregulation of ANK2 expression, suggesting active regulation of the cell structure during MO-to-MAC differentiation. In addition, gene-expression of the chemoattractant chemokine MCP-1, a miR-139-5p molecular-target according the in silico analysis, was significantly up- and down-regulated in MO transfected with miR-139-5p agomiR and antagomiR, respectively, during differentiation to MAC.

Conclusion: Our results suggest an epigenetic regulation through miR-139-5p in human differentiating monocytes, affecting genes involved in cell structure, chemotaxis and inflammation in early stage macrophages.

P091-T | Impact of dietary-induced hypercholesterolemia on HDL-delivery of miRNAs to endothelial cells

S. Ben-Aicha*,†; S. Camino*,†; R. Escate*,†; L. Casani*,†; G. Mendieta‡; T. Padro*,†; L. Badimon*,†; G. Vilahur*,†

**Program ICCC-Institut Català de Ciències Cardiovasculars, IR-Hospital De La Santa Creu I Sant Pau, Barcelona, Spain; †CiberCV, Institute Carlos III, Barcelona, Spain, Barcelona, Spain; ‡Department of Cardiology, Clinic Hospital, Barcelona, Spain*

Introduction: High-density lipoproteins (HDLs) are positively associated with cardiovascular protection and transport miRNAs and deliver them to recipient cells influencing gene expression. We sought to investigate the impact of hypercholesterolemia, a common cardiovascular risk factor, on HDL-bound miRNA profile and miRNA cell transfer.

Methods: Pigs were fed 10 days a normocholesterolemic (NC; $N = 10$) or a hypercholesterolemic (HyC; $N = 10$) diet reaching cholesterol levels of 38·5 (25·8–41·0) mg/dL and 245·5 (166·2–291·2) mg/dL, respectively ($P < 0{\cdot}0001$). HDL particles were isolated from blood samples of all pigs by ultracentrifugation, purified and quantified. We performed a differential HDL-miRNA expression profiling ($n = 149$ miRNAs) between NC- and HyC- HDLs following the multipanel qPCR technique. We also performed culture studies in porcine aortic endothelial cells to determine whether the identified differentially expressed miRNAs were delivered to endothelial recipient cells. Then, by implementing bioinformatic analyses (Ingenuity Pathways Analysis) we identified those functional networks and potential gene targets modulated by the miRNA candidates and further validated the candidates by rt-PCR.

Results: Five microRNAs were differentially expressed between NC- and HyC- HDL (P-value <0·05). Specifically, HyC-HDLs had higher levels of miR-126-5p, miR-126-3p and miR-30b-5p (2·7×, 1·7×, 1·3× respectively) while the levels of miR-103a-3p and let- 7 g-5p were found to be reduced (−1·6×, −1·4×, respectively) vs NC-HDL. Only miR126 (both -3p and -5p) was found to be enhanced in endothelial cells upon HDL treatment. Interestingly, miR-126-3p and -5p levels were found to be 3-fold higher in those endothelial cells incubated with HyC-HDL as compared to NC-HDL ($P < 0·05$), an effect that persisted despite HDL removal and was independent of SRB1 expression. Eighteen top miRNA126-target genes were evaluated being PI3KR2 a potential target gene (P-value < 0·05).

Conclusions: Our results collectively suggest that hypercholesterolemia induces changes in HDL-miRNA signature and enhances HDL-miR126 delivery to endothelial cells likely modulating key processes related with vascular survival and proliferation.

P092-T | Dynamics of nitric oxide production in the rat heart during hypokinesia: effects of inhibitors of NO-synthase L-NAME and aminoguanidine

T. Bogodvid*,†; T. Zefirov*; V. Andrianov*,‡; G. Yafarova*,‡; R. Zaripova*; N. Ziiatdinova*; F. Sitdikov*; V. Iyudin‡; K. Gainutdinov*,‡

*Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russian Federation; †Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russian Federation; ‡Zavoisky Physical-Technical Institute of RAS, Kazan, Russian Federation

The basis of regulation of the cardiac work are sympathoparasympathetic interaction. Currently, a considerable value to realization of regulatory effects contribute by the nitric oxide (NO). There are two main ways of NO production in the body: enzymatic and non-enzymatic. Prolonged hypokinesia causes significant changes in the contractile function of the cardiac muscle. All these phenomena are inevitably lead to a serious deterioration of tissue oxygen supply, i.e. hypoxia. Previously, we carried out the EPR spectroscopic studies of the dynamics of NO production in cardiac and hepatic tissues during hypokinesia of various duration, in which we found a significant increase in NO content on the 30 days of hypokinesia. Therefore, the aim of the study was to investigate the role of NO in the consequences resulting from the hypokinesia by analyzing the NO containing paramagnetic complexes in various tissues of rats which was growing under restricted physical activity.

By the method of EPR spectroscopy it was found an increase in the intensity of production of NO in the rats hearts after 90-days hypokinesia. The nonselective blockade of NO-synthase activity by L-NAME in hypokinesed rats resulted in a decrease of content of NO by 67–70% in atrias and ventricles of the heart. Selective blockade of inducible NO-synthase by aminoguanidine caused a decrease of the content of NO by 60–65% in the tissues of the atrias and ventricles. The obtained results suggested that increasing of NO production under conditions of hypokinesia occurred through the activation of NO-synthase activity.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University (No 17.9783.2017/8.9).

P093-T | A multi-biomarker panel of myocardial remodelling provides incremental prognostic value in heart failure patients

M.U. Moreno*,†,‡; S. Ravassa*,†,‡; C. Gallego§; R. Querejeta¶; B. López*,†,‡; A. Campbell**; A.-M. Jennings††; G.S. José*,†,‡; F.J. Beaumont*,†,‡; S. Padmanabhan‡‡; A.F. Dominiczak‡‡; A. González*,†,‡; J. Díez*,§§; C. Delles‡‡

*University of Navarra, CIMA, Program of Cardiovascular Diseases, Pamplona, Spain; †IdiSNA, Navarra Institute for Health Research, Pamplona, Spain; ‡CIBERCV, Carlos III Institute of Health, Madrid, Spain; §Division of Cardiology, Cardiovid Clinic, Medellín, Colombia; ¶Donostia University Hospital, University of the Basque Country, and Biodonostia Research Institute, San Sebastian, Spain; **Generation Scotland, Centre for Genomic and Experimental Medicine, Institute of Genetics and Molecular Medicine, University of Edinburgh, UK; ††Randox Laboratories, Crumlin, Northern Ireland; ‡‡Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK; §§Department of Cardiology and Cardiac Surgery, University of Navarra Clinic, Pamplona, Spain

Background: Cardiomyocyte injury (CMI), myocardial interstitial fibrosis (MIF) and coronary microvascular endothelial dysfunction and inflammation (EDI) are structural alterations of myocardial remodelling in heart failure (HF). We evaluated the prognostic value of a combination of biomarkers of these alterations in HF patients.

Material and methods: Circulating high-sensitivity troponin-T (hs-TnT), carboxy-terminal propeptide of procollagen type-I (PICP) and carboxy-terminal telopeptide of collagen type-I to matrix metalloproteinase-1 ratio (CITP: MMP-1), and vascular cell adhesion molecule-1 (VCAM-1) as biomarkers of CMI, MIF and EDI, respectively, were measured in HF patients from the Generation Scotland (n = 71) and Leizaran (n = 197) cohorts. The association of their combination with a composite outcome of hospitalization for HF (HHF) or cardiovascular death (CVD) was

examined. Bootstrap and jackknife cross-validation approaches were performed.

Results: CMI (hs-TnT ≥ median), MIF (PICP≥ or CITP: MMP-1 ≤ median) and EDI (VCAM-1 ≥ median) presence was determined in the Generation Scotland patients. Twenty-nine (40·8%), 24 (33·8%) and 18 (25·4%) patients had zero or one, two or three biomarker alterations, respectively, the latter showing higher risk of outcome ($P < 0·05$). A similar median-based categorization confirmed higher risk of outcome ($P < 0·05$) in the Leizaran patients with the three alterations ($n = 50$ [25·4%]). This combination added incremental predictive value as demonstrated by Harrell's *C*-statistic, integrated discrimination and net reclassification improvements ($P \leq 0·04$).

Conclusions: A panel combining biomarkers of CMI, MIF and EDI identifies HF patients at higher risk of HHF or CVD over and beyond established risk factors. These findings reinforce the necessity for a holistic view of myocardial remodelling and hold promise for personalizing risk prediction and treatment of HF patients.

P094-T | Contraction (retraction) of blood clots as a pathogenic factor in thrombosis

A.D. Peshkova*; R.I. Litvinov*,†

**Kazan Federal University, Kazan, Russian Federation; †University of Pennsylvania School of Medicine, Philadelphia, USA*

Background: Contraction or retraction of a blood clot may approximate the wound edges and restore blood flow past obstructive intravascular thrombi. Despite its potential clinical significance, the pathogenic importance of contraction of clots and thrombi remains unknown.

Materials and methods: We studied kinetics of contraction of clots formed from the blood of patients with ischemic stroke (IS) and venous thromboembolism (VTE), including patients with pulmonary embolism (PE). Patients were excluded from this study if they were given antiplatelet drugs at least 2 weeks prior to examination. Functionality of isolated platelets was assessed using flow cytometry and scanning electron microscopy.

Results: The velocity and degree of clot contraction in the blood from patients with IS and VTE was reduced compared to that of healthy subjects. In venous thrombosis associated with PE contraction was significantly impaired compared to the isolated thrombosis, suggesting that decreased clot compaction could increase the risk of embolization. The reduced ability of clots to contract correlated with pronounced platelet dysfunction. Platelets from IS and VTE patients were spontaneously activated as revealed by the more frequent shape change and formation of filopodia in electron micrographs compared to the less common morphologically altered platelets from healthy subjects. In response to chemical stimulation, platelets from the blood of IS and VTE patients had a significantly lower expression of P-selectin and fibrinogen-binding capacity compared to activated normal platelets, suggesting that reduced platelet contractility is a result of exhaustion.

Conclusion: The results suggest that contraction of clots and thrombi is an underappreciated pathogenic mechanism that may affect the course and outcomes of IS and VTE. The clinical importance of reduced clot contraction in IS and VTE as well as the diagnostic and prognostic value of the clot contraction assay is worth further exploration. Work supported by the Program of Competitive Growth of KFU.

P095-T | Vegetative regulation of heart activity in conditions of electrical stimulation of the spinal cord

A. Fedianin; G. Yafarova; A. Militskova; L. Bikchentaeva; T. Baltina

Kazan (Volga region) Federal University, Kazan, Russian Federation

The method of transcutaneous electrical stimulation of the spinal cord (tSCS) is widely used for the rehabilitation of locomotor functions in patients with spinal cord injury. In order to predict undesirable changes in cardiac activity, which can presumably be induced by electric current, it is necessary to take into account the changes in the regulatory mechanisms of the circulatory system. The aim of this work was to study the variability of the heart rate in conditions of transcutaneous electrical stimulation of the spinal cord. The tSCS was performed at the level of T11–12 vertebrae in healthy persons using monophasic rectangular electrical stimuli with duration of 1 ms and frequency of 30 Hz for 1 minute. After the tSCS we observed a decrease in the mean heart rate (M), an increase in the standard deviation (σ). Prior to the tSCS the normotonia of the tested persons was assessed by the mode index (Mo) and the variation in the range (MxDMn). After tSCS the vegetative activity was assessed as a vagotonic. The tSCS did not cause an increase in sympathetic influences: we did not observe an increase in the amplitude of the mode (Amo) and the decreased of the stress index after the tSCS. Thus, the tSCS at the lower thoracic level of stimulation increased the degree of variability of the cardiointervals, which indicates an increase in the tonicity of the parasympathetic system. The results suggested that the application of this method leads to an increase in the processes of self-

regulation and activation of the autonomic circuit regulation of the rhythm of the heart.

This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities no. 17.9783.2017/8.9.

P096-T | Determinants of thrombosis: activation of canonical Wnt pathway

M. Borrell; J. Crespo; E. Peña; L. Badimon

Program ICCC-Institut Català de Ciències Cardiovasculars, IR-Hospital de la Santa Creu i Sant Pau, UAB, CIBERCV, Barcelona, Spain

Background: Platelets are responsible of atherothrombosis. They can interact with different proteins and coagulation factors in very complex ways to generate thrombus. The activation of platelets intracellular signaling pathways defines the fate of the event, and the study of the different signaling pathways may help to separate thrombosis from bleeding. We aimed to define the role of LRP5 and the canonical Wnt pathway in platelet function.

Materials and methods: Platelet deposition, thrombus formation, platelet adhesion and platelet secretion were studied in blood from Wt and Lrp5 depleted mice. Downstream signaling of the collagen receptors GPVI, α2β1 and FcRγ and of the ADP receptors P2Y12, P2Y1 and P2X1 were analyzed in platelets of Wt and $Lrp5^{-/-}$ mice.

Results: Platelet deposition on collagen coated surfaces in Wt and $Lrp5^{-/-}$ mice was significantly different. Platelets from $Lrp5^{-/-}$ animals had a highly reduced deposition with respect to Wt mice. Platelet GPVI protein expression was lower in $Lrp5^{-/-}$ mice than in Wt platelets. However, GPVI downstream signaling after collagen stimulation showed no differences in platelets from Wt and $Lrp5^{-/-}$ mice. ADP platelet stimulation induced a reduction in platelet release of α- and dense granules in $Lrp5^{-/-}$ mice with respect to Wt mice platelets. Platelet P2Y12 receptor levels were significantly lower in $Lrp5^{-/-}$ mice as well as VASP phosphorylation confirming that the downstream signaling pathway of P2Y12 is downregulated in $Lrp5^{-/-}$ mice.

Conclusion: Our data demonstrate that LRP5 regulates thrombosis because genetic deletion of LRP5 reduces the thrombotic potential of platelets. Activation of the canonical Wnt pathway acts as a positive regulator of platelet function in vitro and in vivo.

P097-T | The human heart post-translational proteome: modified proteins as markers of aging and type 2 diabetes in the context of cardiovascular disease

M. Gómez-Serrano*; I. Jorge*,†; C. Castans*; N. Bagwan*; E. Bonzón-Kulichenko*,†; I.S. de Puerta*; M. Ruiz-Meana†,‡; D. García-Dorado†,‡; J. Vázquez*,†

**Spanish Centre for Cardiovascular Research (CNIC) – Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain; †Centro de Investigación Biomédica en Red sobre Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; ‡Department of Cardiology, Vall d'Hebron University Hospital and Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain*

Background: Cardiovascular disease (CVD) is the leading cause of death in the world. Its increase has been exacerbated by the combined effect of aging, lifestyle changes and the epidemic rising of obesity and type 2 diabetes (T2DM) during the last years. A growing body of evidence indicates that modified proteins are the main effectors in metabolic and CVD as well as important sensors of damage and cellular stress suggesting that post-translational modifications (PTMs) could potentially be used as biomarkers.

Material and methods: Here, we have evaluated the complete landscape of PTMs in protein extracts of the heart right atrial appendage from a set of individuals matched by age, CVD and T2DM clinical history ($n = 31$). We have resorted to mass spectrometry (MS) approaches using an innovative algorithm (Comet-PTM) for its analysis. This ad-hoc algorithm bypasses the main limitations of current open search approaches allowing a comprehensive analysis of all PTMs detectable by MS and the simultaneous quantification of them and their belonging proteins.

Results: Our preliminary analysis has identified near 100 000 modified peptides in the human heart proteome. Our results highlight the significant abundance of PTMs such as oxidations, methylations, phosphorylations, deamidations, carbonylations or advanced glycation end-products (AGEs), highlighting an important role of these modifications in aging, T2DM and CVD. Particularly, we have uncovered more than 1000 significantly modified peptides with aging ($P < 0{\cdot}05$). Interestingly, some of these PTMs belong to proteins previously implicated in cardiovascular and metabolic homeostasis like the natriuretic peptide (NPPA) as well as glycolytic, mitochondrial or collagen proteins.

Conclusions: We have characterized the comprehensive landscape of modifications in the human heart proteome. Our findings advance in the understanding of the mechanisms underlying physiological aging and its interplay with CVD and T2DM, and pave the way towards the use of PTMs as novel biomarkers in the clinical setting.

P098-T | Hallmarks of early damage upon ischemia/reperfusion in a pig myocardial infarction model

I. Jorge*,†; A. Binek*; R. Fernández-Jiménez*,†,‡; C. Castans*; C. Galán-Arriola*,†; E. Camafeita*,†; A. Pun*,†; B. Ibáñez*,†,§; J. Vázquez*,†

**Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; †CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; ‡The Zena and Michael A. Wiener CVI, Icahn School of Medicine at Mount Sinai, New York, USA; §IIS-Fundación Jiménez Díaz Hospital, Madrid, Spain*

Background: Despite that both the ischemia and reperfusion are known to contribute to myocardial infarction (MI) injury, the molecular mechanisms implicated in early heart damage upon cardiac insult remain to be elucidated. The present study aims at identifying proteins and post-translational modifications (PTMs) thereof that could be used as biomarkers of MI damage. For that, we have tackled proteome dynamics in the ischemic and remote myocardium after ischemia/reperfusion (I/R) as well as the impact of experimental pre-conditioning (PC) strategy in a clinically-relevant pig model.

Material and methods: Closed-chest 40 minutes ischemia followed by reperfusion was performed in groups of 5 pigs sacrificed at 120 minutes, 24 hours, 4 days and 7 days after reperfusion, as well as at 24 hours in PC groups. Additionally, 5 healthy pigs served as controls. Myocardial tissue samples were processed to high-resolution mass spectrometry-based proteomics analysis.

Results: Inflammation-related proteins increased in the ischemic area post-I/R. In addition, proteins involved in energetic metabolism processes, junction membrane complex and sarcomere structure decreased upon reperfusion. The majority of these proteins reverted to control levels in 24 hours PC pig group. Likewise, the transient decrease in contractile and mitochondrial proteins observed in the remote myocardium early after I/R was not observed in pigs subjected to PC.

In the ischemic myocardium, a peak of cysteine reversible oxidation and other irreversible oxidative PTMs, mainly affecting mitochondrial, contractile and extracellular matrix proteins, was observed between 120 minutes and 24 hours post-I/R. This oxidative damage coincided with an increase in leukocyte migration and adhesion proteins, suggesting neutrophil infiltration to the lesion site, and was attenuated upon PC treatment.

Conclusions: This study reveals new clues in the molecular mechanisms underlying MI in both ischemic and remote myocardium after I/R, providing candidate biomarkers of early myocardial damage that can contribute to the development of new therapeutic treatments aiming at cardiac injury prevention.

P099-T | Modulation of GRK2 levels and functionality in myocardial ischemia/reperfusion and impact in the regulation of cardioprotective signaling pathways

P. Penela*; P. Ramos*; J. Inserte†; D. Gracía-Dorado†; F. Mayor*

**Departamento de Biología Molecular, Universidad Autónoma de Madrid y Centro De Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain; †Servicio de Cardiologia, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain*

Background: G protein-coupled receptor kinase 2 (GRK2) modulates cardiac G protein-coupled receptors (GPCRs) and is also a node in additional signalling pathways. GRK2 is up-regulated in patients and in experimental models of heart failure, and its inhibition is cardioprotective in the long-term in mice.

Objective: Assess whether GRK2 levels are altered at early time points following myocardial ischemia/reperfusion (I/R) and its functional impact.

Methods: We investigated the temporal changes of GRK2 and their consequences on the cardioprotective AKT pathway in rat experimental models of I/R in the context of pre-conditioning treatments.

Results: Ischemia promotes enhanced phosphorylation of GRK2 on residues known to trigger proteasome-dependent degradation, while during reperfusion the oxidant-induced activation of PKA leads to phosphorylation of GRK2 on S685 and its degradation by calpain. Concurrent to GRK2 downmodulation, a marked loss of total AKT protein is noted in parallel with a deficient AKT-mediated substrate phosphorylation, despite the hyper-activation of the remaining AKT protein, suggesting that pro-survival AKT signaling fails during I/R. We uncover that I/R promotes the hyper-acetylation and protein decay of Pin1, a positive regulator of AKT function and stability. Interestingly, in contrast to preconditioning which triggers a partial recovery of AKT levels and functionality, calpain inhibition allows protein normalization of Pin1, AKT and GRK2 and improves myocardial functionality.

Conclusions: We hypothesize that PKA-mediated degradation of GRK2 during I/R impairs positive GRK2-modulated HDAC6 activity towards Pin1 and AKT functionality and reduces cardioprotection, and that these effects might be attenuated by the combined inhibition of calpains and proteasome

P100-T | Role of NDUFAL2 in basal heart tolerance against isquemia-reperfusion injury

D. Tello*,†; Q.O.Y. Li*,†; M. Ruiz-Meana†,‡,§; J. Inserte†,‡,§; D. Garcia-Dorado†,‡,§; J. Aragonés*,†

**Research Unit, Hospital of Santa Cristina, Research Institute Princesa (IP), Autonomous University of Madrid, Madrid 28009, Spain; †CIBER de Enfermedades Cardiovasculares, Carlos III Health Institute, Madrid, Spain; ‡Department of Cardiology, Universitat Autònoma de Barcelona, Barcelona, Spain; §Laboratory of Experimental Cardiology, Universitat Autònoma de Barcelona, Barcelona, Spain*

Background: NDUFA4L2 is target gene of hypoxia-inducible factors (HIF) that adapt cells to low oxygen tension by decreasing overall mitochondrial metabolism through the inhibition of mitochondrial complex I. Along this line, a crucial feature that makes NDUFA4L2 one of the most relevant proteins during cell adaptation to hypoxia is its ability to diminish the production of reactive oxygen species (ROS) in mitochondria, which is essential for cell protection against oxidative damage. Thus, although the protective role of NDUFA4L2 in in vitro scenarios where ROS are important for cell viability is well known, it is also crucial to know the pathophysiological role of NDUFA4L2 in vivo.

Material and methods: To investigate the role of NDUFA4L2 in vivo we generated a NDUFA4L2 deficient mice (C57BL/6) in which the exon 2 was flanked by LoxP sites and then excised by the recombinase Ub-CRERT2 in the germ line to obtain a constitutive and global deletion of NDUFA4L2. Then, the relative abundance of NDUFA4L2 at mRNA and protein level in different tissues was tested, as well as its ability to protect against heart ischemia-reperfusion damage using the Langendorff model (35′ ischemia following 60′ reperfusion) and assessing infarct size by triphenyl tetrazolium reaction.

Results: Our data show that the heart and the lung are the organs in which NDUFA4L2 presents the highest expression at mRNA and protein level. Moreover, NDUFA4L2 deficient mice exhibited a higher lactate dehydrogenase release into the effluent during reperfusion after transient ischemia as well as a reduction of infarct size, which therefore identify a role of NDUFA4L2 in the cardiac tolerance against ischemia-reperfusion insult.

Conclusions: NDUFA4L2, a mitochondrial regulator highly expressed in heart tissue, is essential to control baseline heart protection against ischemia-reperfusion damage and therefore could be a potential therapeutic target in scenarios in which ROS-induced cardiac injury is involved.

P101-T | The mitochondrial antioxidant MitoQ improves the metabolic consequences associated with obesity

G. Marín-Royo*; A.L. Pape*; R. Jurado-López*; E. Martínez-Martínez*; C. Rodríguez†; J. Martínez-González‡; V. Cachofeiro*

**Departamento De Fisiología, Facultad De Medicina, Ucm, Madrid, Spain; †Institut de Recerca del Hospital de la Santa Creu i Sant Pau-Programa ICCC, IIB-Sant Pau, Barcelona, Spain; ‡Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), IIB-Sant Pau, Barcelona, Spain*

Background: Obesity is a pathological situation accompanied with metabolic alterations as consequence of white adipose tissue (WAT) modifications. Multiple mechanisms have been proposed as the trigger of WAT remodelling, including oxidative stress, with the mitochondria being the main source of reactive oxygen species. However, the role played by mitochondrial oxidative stress in the WAT remodelling and metabolic consequences of obesity remains unclear. Therefore, we evaluated the impact of the administration of a mitochondrial antioxidant in the metabolic alterations and the adipose tissue remodelling associated with obesity.

Materials and methods: Male Wistar rats were fed either a high fat diet (HFD, 35% fat, $n = 16$) or a standard diet (CT, 3·5% fat, $n = 16$) for 6 weeks. Half of the animals of each group received the mitochondrial-targeted antioxidant MitoQ (50 mg/kg/day) in the drinking water.

Results: The administration of MitoQ was able to partially prevent the increase in body weight, adiposity index, HOMA and adipose tissue remodelling in HFD rats. MitoQ also ameliorated the alterations in factors involved in insulin signalling observed in obese rats: the reduction in protein levels of adiponectin and GLUT4, GLP1 and the increase in those of DDP4, SOCS3 and phosphorylation of IRS-1. Obesity was also associated with alterations in proteins levels involved in mitochondrial function: increased levels of cyclophilin F and carnitine palmitoyl transferase 1A (CPT1A) and reduced levels of mitofusin1, PEROXYREDOXIN IV and fumarase. These changes were improved by the mitochondrial antioxidant.

Conclusions: In conclusion, these data show that treatment with the mitochondrial antioxidant MitoQ was able to improve the metabolic alterations associated with obesity. This beneficial effect was accompanied by the improvement of WAT remodelling and mitochondrial function, suggesting that mitochondrial dysfunction plays an important role in the metabolic consequences in the context of obesity.

P102-T | Arylesterase – prognostic factor for 1-year reinfarction in patients with acute myocardial infarction

L. Ciumarnean*; A. Macarie†; M. Greavu‡; M. Milaciu*; T. Moldovan§; S. Vesa¶

*Department of Internal Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; †Department of Geriatrics and Gerontology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; ‡Department of Internal Medicine, "Transilvania" University, Brasov, Romania; §Oncology Institute "Prof. Dr. Ion Chiricuță", Cluj-Napoca, Romania, Cluj-Napoca, Romania; ¶Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Background: The purpose of the study was to evaluate the prognostic value of serum paraoxonase 1 activities (lactonase, arylesterase and paraoxonase) for 1-year reinfarction risk in patients with acute myocardial infarction (MI).

Materials and methods: The study included 75 patients (mean age 64·4 ± 12·5 years; 25 [33·3%] women and 50 [66·7%] men) which were diagnosed with acute MI, using the criteria in place. All patients underwent coronarography. We determined the PON1 activities (paraoxonase, arylesterase and lactonase) by spectrophotometric methods in heparinized plasma. We did a 1-year follow-up and recorded the rate of reinfarction in these patients.

Results: There were 23 (30·7%) patients that presented reinfarction. The paraoxonase values were higher in those without complications (26·6 [17·3; 51·6 U/mL]) than in patients with reinfarction (23·5 [14·1; 59·8] U/mL) (P = 0·5). The lactonase levels were higher in those without complications (27·2 [19·7; 33·4 μM/L]) than in patients with reinfarction (23 [19·6; 28·2 μM/L]) (P = 0·1). The arylesterase levels were higher in those without complications (13·8 [10·5; 16·7 U/mL]) than in patients with reinfarction (11·7 [9·1; 13·9 U/mL]) (P = 0·05). Arylesterase levels higher than 12·3 U/mL were associated with fewer reinfarctions at 1 year after a MI (OR, 0·23 [95% CI 0·08–0·7; P = 0·01]). The reinfarction was not predicted by the type of infarction, type of intervention, or medication taken by the patients.

Conclusions: The study showed that elevated levels of arylesterase were associated with fewer reinfarctions at 1 year after an acute MI.

P103-T | VKORC1-1639 G>A polymorphism and the risk of vascular calcification. A prospective study

S. Vesa*; M. Sava†; A. Trifa‡; S. Crisan§; A. Buzoianu*

*Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; †Department of Dermatology, University of Oradea, Oradea, Romania; ‡Department of Medical Genetics, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; §Department of Internal Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Background: Vitamin K deficiency can induce vascular calcifications, through a decrease of the expression of vitamin K dependent proteins. The aim of the study was to determine whether the presence of a polymorphism in the gene encoding vitamin K epoxide reductase (VKOR) enzyme can increase the risk of vascular calcifications in patients receiving long-term treatment with acenocoumarol.

Material and methods: The study included 59 patients without carotid plaques diagnosed with atrial fibrillation or deep vein thrombosis that required long-term anticoagulation with acenocoumarol and 17 controls without carotid plaques and treatment with acenocoumarol. The following parameters were noted: age, gender, comorbidities (arterial hypertension, ischemic heart disease, heart failure, dyslipidemia, obesity, type 2 diabetes). Genetic analysis included genotyping for the VKORC1-1639 G>A mutation. The patients were followed for 3 years and the presence of carotid was noted.

Results: The calcified carotid plaques were found in 15 (19·7%) patients after 3 years of follow-up. The GG genotype of VKORC1 -1639 G>A polymorphism was present in 3 (20%) patients with carotid plaques and in 25 (41%) patients without plaques; the GA genotype was present in 7 (46·7%) patients with carotid plaques and in 29 (47·5%) patients without plaques; the AA genotype was present in 5 (33·3%) patients with carotid plaques and in 7 (11·5%) patients without plaques (P = 0·07). The GG + GA genotypes were present in 10 (66·7%) patients with carotid plaques and in 54 (88·5%) patients without plaques (P = 0·05). The obesity, ischemic heart disease and dyslipidemia were associated with the presence of carotid plaques (P = 0·06; P = 0·04). The treatment with acenocoumarol and the other variables were not statistically significant linked to carotid plaques.

Conclusion: The AA genotype, obesity, ischemic heart disease and dyslipidemia could be associated with calcified carotid plaques.

P104-T | Automated ECG segmentation based on weighted reference shape updating

A. Sokolova*; Y. Uljanitski*; M. Bogachev*,†

*St. Petersburg Electrotechnical University, St. Petersburg, Russian Federation; †Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russian Federation

Background: Stress-test conditions characterized by rapid changed in ECG morphology and high noise levels strongly limit simple ECG shape analysis methods encouraging the use of noise robust approaches based on cosine similarity scores (CSS) with reference ECG shapes.

Material and methods: 18 short-term ECG recordings during Harvard step testing were obtained in-house and subjected to the automated ECG segmentation by finding the local maxima of the CSS ρ given that $\rho > 0{\cdot}9$ for QRS and $\rho > 0{\cdot}7$ for PQRST shapes. 18 long-term ECG recordings obtained from the MIT-BIH Normal sinus rhythm database without abrupt changes of ECG shape and/or heart rate were used as control. Neither of the records contained any significant arrhythmias or ECG shape morphology anomalies.

Results: QRS detection with reference shape obtained by averaging of the previous 10 detected QRS shapes performed well for the control records ($FN = 9{\cdot}2{\cdot}10^{-3}$), while appeared completely inadequate for stress-test conditions ($FN = 0{\cdot}41$), $FP < 10^{-5}$ in all cases here and thereafter. In contrast, similar analysis with reference QRS obtained as the sum of the previous reference QRS shape with weight ρ and the last detected QRS shape with weight $1 - \rho$ exhibited good performance for both control ($FN = 7{\cdot}6{\cdot}10^{-3}$) and stress test ($FN = 3{\cdot}4{\cdot}10^{-4}$) conditions. Moreover, replacement of the reference shape by its best quadratic fit led to the lower $FN = 1{\cdot}8{\cdot}10^{-3}$. Since most of the false alarms during QRS detection occurred at T-waves, we also tested PQRST detection and obtained $FN = 3{\cdot}78{\cdot}10^{-3}$ and $FN = 3{\cdot}34{\cdot}10^{-3}$ for the updated shape and its quadratic fit, respectively.

Conclusion: Our results indicate that the introduction of the weighted reference shape updating improved the automated ECG segmentation considerably, especially under stress test conditions where the conventional approach failed. The study was supported by the Ministry of Education and Science of Russia (2.5475.2017/6.7) and by the program of the competitive development of Kazan Federal University.

P105-T | Identification of early peripheral blood biomarkers to predict the severity of myocardial infarction in a porcine model

J. García-Casado*,†; R. Blázquez*,†; V. Álvarez*; J.A. Antequera-Barroso‡; C. Báez-Díaz*,†; V. Blanco*; J. Maestre*; B. Moreno*; E. López*; F. Marinaro*; V. Crisóstomo*,†; F.M. Sánchez-Margallo*,†

*Minimally Invasive Surgery Centre, Cáceres, Spain; †CIBER de Enfermedades Cardiovasculares, Madrid, Spain; ‡Mathematics and Experimental Sciences Department, University of Extremadura, Cáceres, Spain

Background: The porcine model has a great relevance as a preclinical model in cardiovascular medicine. It is widely used to understand the biological mechanism involved in cardiovascular disorders and to evaluate new therapeutic products. In this sense, different closed-chest models of myocardial infarction have been successfully developed in the last years to mimic the pathophysiological events in humans. To deeply characterize this porcine myocardial infarction model, this work aimed to study the distribution of different lymphocyte subpopulations following the induction of the infarction. Moreover, peripheral blood leukocytes were correlated with functional and biochemical parameters which could be used as biomarkers for the severity of myocardial infarction.

Material and methods: Fourteen Large White pigs were subjected to a closed chest myocardial infarction by balloon inflation during 90 minutes. Peripheral blood leukocytes were analyzed before the myocardial infarction and one hour after. Lymphocytes were isolated and a phenotypical analysis was performed by flow cytometry. One week after myocardial infarction induction, serum levels of cardiac enzymes (troponin I and CK-MB) and cardiac output measurements obtained by MRI (infarct area and ejection fraction) were determined.

Results: Our results revealed a significant increase of $CD4^+$ T cells, $CD4^+/CD8^+$ ratio and naïve $CD8^+$ T cells ($CD8^+$ $CD27^+$ $CD45RA^+$) at one hour post-infarction when compared to pre-infarction levels. More importantly, we found a significant correlation between cardiac enzymes with T cell subsets: $CD4^+$ T cells and naïve $CD8^+$ T cells.

Conclusions: The analysis of peripheral blood leukocytes at one hour post-myocardial infarction could be used as early and affordable biomarkers to predict the severity of the myocardial infarction in this animal model.

P106-T | Isolation and proteomics analysis of microvesicles from cardiosphere-derived cells for cardiac repair therapy

J. García-Casado*,†; E. López*; V. Álvarez*; R. Blázquez*,†; F. Marinaro*; M. Gómez-Serrano‡; I. Jorge†,‡; J. Vázquez†,‡; F.-M. Sánchez-Margallo*,†

**Minimally Invasive Surgery Centre, Cáceres, Spain; †CIBER de Enfermedades Cardiovasculares, Madrid, Spain; ‡Cardiovascular Proteomics Lab, Centro Nacional Investigaciones Cardiovasculares (CNIC), Madrid, Spain*

Background: The cardiosphere-derived cells (CDCs) have emerged as an effective approach for cardiovascular cell therapy. These cells have an immunomodulatory effect providing an optimal microenvironment for cardiac repair. Microvesicles derived from CDCs (MV-CDCs) reduce the apoptosis and enhance the angiogenesis being crucial for the regeneration of injured heart muscle. Preclinical studies in porcine models have demonstrated that these exosomes attenuate adverse remodeling improving left ventricular function. Here we hypothesize that the therapeutic potential of MV-CDCs is closely dependent on in vitro CDCs preconditioning. The aim of this work was to identify potential biomarkers that could predict the therapeutic efficacy of these microvesicles.

Material and methods: Porcine CDCs were isolated and characterized by FACS. The MV-CDCs were characterized by surface markers and Field-Flow Fractionation. Protein extracts were processed by high-resolution liquid chromatography coupled to mass spectrometry-based proteomic analyses.

Results: The characterization of CDCs showed a positive expression for CD29, CD44, CD90, CD117, Sca-1 and SLA-I markers and negative expression for CD31, CD45, CD61 and SLA-II markers. Field-Flow Fractionation analysis detected particles with a 198 nm of diameter and an estimated molecular weight of $1{\cdot}56 \times 10^5$ kDa. A total of 759 proteins (with more than two peptides per protein at 1% FDR) were identified in these microvesicles revealing a significant over-representation of the extracellular exosome (GO: 0070062, $P < 0{\cdot}01$, 5% FDR) according to the DAVID functional annotation database. Of note, we detected 95 out of the 100 top identified proteins in ExoCarta database. Additionally, enrichment analyses revealed that MV-CDCs proteins were related to biological processes such as collagen fibril organization (GO: 0030199), angiogenesis (GO: 0001525) or platelet aggregation (GO: 0070527) among others.

Conclusions: In this study, we present the first in-depth characterization of the MV-CDCs protein profile, providing new candidate biomarkers that might serve to predict the therapeutic potential of pre-conditioned MV-CDCs in the upcoming studies.

P107-T | Impact of coronary revascularization in patients undergoing TAVI

A.A. Carrillo; D.L. Otero; R.T. Nouche; J.C.S. Pena; A.B.C. Álvarez; F.G. Peña; A.R. Diéguez; J.R.G. Juanatey

Hospital Universitario De Santiago De Compostela, Santiago De Compostela, Spain

Background: Patients who undergo transcatheter aortic valve implantation (TAVI) frequently have concomitant coronary artery disease (CAD) with the need for revascularization, unknowing how complete that must be.

Material and methods: From November 2008 to August 2016, the baseline SYNTAX Score (bSS) and the SYNTAX Residual Score (rSS) were calculated before to TAVI; we evaluated the predictive value of coronary artery disease and the coronary revascularization according to the rSS, dividing into 3 groups: complete revascularization (RC) ($rSS = 0$), reasonable incomplete revascularization (RRI) ($0 > rSS < 8$) and incomplete revascularization (RI) ($rSS \geq 8$), in the occurrence of (MACE) (any cause of mortality, myocardial infarction, not planned revascularization) and heart failure (HF). Estimated predicted event rates were obtained from the Cox regression models and Kaplan–Meier survival curves, these were made without adjustment and subsequently adjusted by STS and Euroscore II.

Results: 349 patients were included, with a mean age of $82{\cdot}4 \pm 5{\cdot}7$ years, 53% were women, 187 patients (53·6%) had CAD, of these, 56 patients (29·9%) had CR, 85 (45·4%) had RRI and 46 (24·5%) had RI. The mean of bSS was $9{\cdot}2 \pm 8{\cdot}1$, with a mean follow-up of $35{\cdot}2 \pm 25{\cdot}3$ months; no differences were observed in the occurrence of MACE between the groups of CAD vs No CAD ($P = 0{\cdot}906$), or between the different degrees of revascularization ($P = 0{\cdot}866$), no difference was observed in the admissions by HF ($P = 0{\cdot}748$), without worsening the risk of MACE ($P = 0{\cdot}971$) when they were added to it, even when were adjusted by STS or EuroScore II.

Conclusions: The CAD and the degree of revascularization in patients undergoing TAVI were not associated with an increase in the frequency of MACE or admissions for HF in the long-term follow-up.

P108-T | Validation of engineered cardiac grafts for the local delivery of multifunctional extracellular vesicles for myocardial repair

S. Roura*,†; M.M. Monguió-Tortajada*,‡,§; C. Prat-Vidal*,†,¶; C. Gálvez-Montón*,†; F.E. Borràs‡,§,**; A. Bayes-Genis*,†,††,‡‡

*ICREC Research Program, IGTP, Badalona, Spain; †CIBERCV, Instituto de Salud Carlos III, Madrid, Spain; ‡REMAR-IVECAT Group, IGTP, Badalona, Spain; §Department of Cell Biology, Physiology and Immunology, UAB, Barcelona, Spain; ¶Center of Regenerative Medicine in Barcelona, Barcelona, Spain; **Nephrology Service, Germans Trias i Pujol University Hospital, Badalona, Spain; ††Cardiology Service, Germans Trias i Pujol University Hospital, Badalona, Spain; ‡‡Department of Medicine, UAB, Barcelona, Spain

Background: The administration of extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) is a promising alternative treatment for several pathologies, including cardiac repair after myocardial infarction (MI). EVs have been shown to display biological properties of the originating cells and thus, MSC-EVs have immunomodulatory, regenerative and pro-angiogenic capabilities both autologous and allogeneically. However, the optimal delivery strategy for EV therapy remains challenging. One of the possibilities is the use of novel bioengineered 3D scaffolds as an efficient support for the local delivery of bioactive, multifunctional EVs.

Material and methods: EVs were purified from porcine cardiac adipose tissue MSCs by size exclusion chromatography and were characterized morphologically and phenotypically. Two decellularized cardiac scaffolds were then developed from myocardial and pericardial tissues and were embedded with fluorescently-labelled MSC-EVs for tracking and retention assessment.

Results: The regenerative, alloreactivity and immunomodulatory properties of porcine MSC-EVs were assessed in vitro to validate their potential for myocardial repair. The structure of the two acellular scaffolds was preserved upon the decellularization process and their proteome characterization showed enrichment of matrisome proteins and major cardiac extracellular matrix components. Both engineered cardiac scaffolds retained MSC-EVs even after thorough washing and a week-long culture, as shown by whole-tissue fluorometric scanning, confocal and scanning electron microscopy imaging.

Conclusions: Collectively, our data indicate that both engineered cardiac scaffolds may be suited for effective EV local administration and will be further evaluated in preclinical MI swine models on restoring cardiac function post-MI. The confined administration of multifunctional EVs within a scaffold may potentiate cardiac repair by increasing the local dose of MSC-EVs, constitute a bioactive niche for regeneration, and could be used as a cell-free, off-the-shelf product to regenerate post-infarcted myocardium.

P110-T | Orosomucoid 1 in familial hypercholesterolemia

R. Aledo*,†; T. Padro*,†; P. Mata‡; O. Muñiz§; M. Borrell*,†; L. Badimon*,†

*Program ICCC-Institut Català de Ciències Cardiovasculars, IR-Hospital de la Santa Creu i Sant Pau, UAB, Barcelona, Spain; †CiberCV, Institute Carlos III, Barcelona, Spain, Barcelona, Spain; ‡Fundación Hipercolesterolemia Familiar, Madrid, Spain; §Hospital Virgen del Rocio, Sevilla, Spain

Background: Heterozygous Familial hypercholesterolemia (FH) is a genetic disorder characterized by high levels of low-density lipoprotein (LDL) in the blood that can lead to premature coronary heart disease. Accumulation of LDL in the vascular wall triggers atherosclerosis with activation of the innate immunity system. We have recently shown that innate immunity cells in FH patients (treated to guidelines) have reduced expression of the anti-inflammatory receptor CD163 indicating less atheroprotection. Here we aim to investigate whether ORM1 expression in peripheral blood leukocytes (PBL) of FH patients is related to the presence of atherosclerotic plaques detected by magnetic resonance imaging (MRI).

Materials and methods: 31 heterozygous FH patients (17 men, 14 women, 25–65 years) belonging to the Spanish FH Longitudinal Cohort Study (SAFEHEART) had their atherosclerotic burden analyzed by MRI. Total mRNA was obtained from PBLs and ORM1 gene expression was determined. α1-acid glycoprotein-1 (AGP), the protein encoded by the ORM1 gene, was analyzed in plasma levels by ELISA. To evidence presence of ORM1/AGP in atherosclerotic plaques, coronary arteries of non-FH transplanted hearts were investigated by immunohistochemistry.

Results: MRI analyses showed different atherosclerotic lesions stages: 21 subjects had fibrous plaques, 8 subjects showed lipid-rich plaques and 2 individuals did not have any plaques. ORM1 was significantly over-expressed in PBL of patients with lipid-rich atherosclerotic plaques while the level of expression was lower in FH individuals with no plaque as well as in FH subjects with fibrotic plaques. AGP plasma levels were increased in FH subjects with fibrotic and lipid rich plaques. IHC analyses of atherosclerotic plaques showed AGP staining only in lipid-rich atherosclerotic plaques.

Conclusions: High ORM1 expression levels in PBL correlate with lipid-rich plaques in FH patients suggesting that the life-time exposure to LDL induces an inflammatory phenotype in innate immunity circulating cells, in plasma and in lipid-rich atherosclerotic plaques.

P111-T | MicroRNA-145 regulates the differentiation of adipose stem cells toward endothelial cells by ETS1 expression, and promotes angiogenesis in vivo

G. Arderiu*; E. Peña*,†; R. Aledo*,†; J. Crespo*; G. Vilahur*,†; L. Badimon*,†

*Program-ICCC, Institut de Recerca Sant Pau, IIB-Sant Pau, Barcelona, Spain; †Ciber CV, Instituto Carlor III, Madrid, Spain

Background: Adipose-derived stem cells (ASCs) are a potential adult mesenchymal stem cell source for restoring endothelial function in ischemic diseases. However, the factors that promote ASCs differentiation toward endothelial cells (ECs) are not well defined. Recent studies have demonstrated that miRNA play a role in cell differentiation. miR-145 has been found to be involved in cell differentiation and vessel stabilization.

Objective: To evaluated the effects of ECs on the proliferation, migration, and potential differentiation of ASCs through miRNA-145 expression.

Methods and results: ASCs were isolated from clinical lipoaspirates and cultured with DMEM or endothelial conditioned medium (ECM). ECM induced downregulation of miR-145 in ASCs and promoted endothelial functional responses. bFGF released by EC was identified as responsible for decreasing miR-145 in ASCs. Modulation of miR-145 in ASCs, using a miR-145 inhibitor, induced EC differentiation, increasing endothelial cell markers as VE-cadherin, VEGFR2 or VWF, proliferation, migration and tube-like formation in in vitro, and enhanced angiogenesis in in vivo Matrigel plugs implanted in mice. miR-145 knockdown in ASCs resulted in a significant increase of ETS1 expression. Upregulation of miR-145 in ASCs, by mimic miR-145, suppress ETS1 expression and consequently EC differentiation and angiogenic properties of ECM-preconditioned ASCs, whereas that overexpression of ETS1 reverse the abrogated anti-angiogenic capacity of miR-145 expression in ASCs. ETS1 overexpression induced similar results to those obtained in miR-145 knockdown experiments.

Conclusions: ECM induces ASCs to EC differentiation and vascular network formation through miR-145-regulated expression of ETS1. These results provides the framework for the development of a potentially novel approach for therapy utilizing ASCs in the treatment of ischemic diseases.

P112-T | Neutrophil gelatinase-associated lipocalin from immune cells is mandatory for aldosterone-induced cardiac remodeling and inflammation

E. Martínez-martínez*; M. Buonafine*; C. Amador†; B. Gravez*; J. Ibarrola‡; A. Fernández-Celis‡; S.E. Moghrabi*; P. Rossignol§; N. López-Andrés‡; F. Jaisser*

*Inserm 1138, Centre De Recherche Des Cordeliers, Paris, France; †Centro de Investigación Biomédica, Universidad Autónoma de Chile, Santiago de Chile, Chile; ‡Cardiovascular Translational Research, Navarrabiomed, Complejo Hospitalario de Navarra (CHN), Universidad Pública de Navarra (UPNA), Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain; §INSERM, Clinical Investigation Centre 1433, French-Clinical Research Infrastructure Network (F-CRIN) INI-CRCT, Nancy, France

Background: Immune system activation is involved in cardiovascular (CV) inflammation and fibrosis, following activation of the mineralocorticoid receptor (MR). We previously showed that Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a novel target of MR signaling in CV tissue and plays a critical role in aldosterone/MR-dependent hypertension and fibrosis. We hypothesized that the production of NGAL by immune cells may play an important part in the mediation of these deleterious mineralocorticoid-induced effects. We analyzed the effect of aldosterone on immune cell recruitment and NGAL expression in vivo.

Material and methods: We then studied the role of NGAL produced by immune cells in aldosterone-mediated cardiac inflammation and remodeling using mice depleted for NGAL in their immune cells by bone marrow transplantation and subjected to mineralocorticoid challenge NAS (Nephrectomy, Aldosterone 200 μg/kg/day, Salt 1%).

Results: NAS treatment induced the recruitment of various immune cell populations to lymph nodes (granulocytes, B lymphocytes, activated $CD8^+$ T lymphocytes) and the induction of NGAL expression in macrophages, dendritic cells, and PBMCs. Mice depleted for NGAL in their immune cells were protected against NAS-induced cardiac remodeling and inflammation.

Conclusions: We conclude that NGAL produced by immune cells plays a pivotal role in cardiac damage under mineralocorticoid excess. Our data further stressed a pathogenic role of NGAL in cardiac damages, besides its relevance as a biomarker of renal injury.

P113-T | Genetic testing to guide warfarin dosage in Russian Federation

A. Ananeva*; L. Shigapova*; A. Nikitin†; O. Gusev*,‡; E. Shagimardanova*

*Kazan (Volga Region) Federal University, Kazan, Russian Federation; †Federal Research and Clinical Center, FMBA, Moscow, Russian Federation; ‡RIKEN, Yokohama City, Japan

Background: Warfarin is widely prescribed anticoagulant in thromboembolic disorders, that acts by reducing the activity of vitamin K-dependent clotting factors. The dose of warfarin should be adapted for each patient, based on individual properties and measurements: weight, height, ethnic background and presence of SNPs in genes involved in warfarin metabolism. In this study, we aimed to determine the frequency of clinically relevant SNP in CYP2C9 and VKORC1 in population of Tatarstan region in Russian Federation.

Material and methods: DNA isolated from blood sample of 158 people was used for library construction and CYP2C9 and VKORC1 genes enrichment. Targeted gene enrichment was performed using NimbleGen SeqCap EZ Choice (Roche) according to the manufacturer's instructions with further sequencing using an Illumina MiSeq instrument with read length 249 bp from each end of the fragment.

Results: Among 158 people, 25 (15.8%) CYP2C9*2 and 29 (18%) CY2PC9*3 SNPs were detected (total 34%). Seven out of 158 (4.4%) respondents had other SNPs, which is known to affect warfarin metabolism. In addition, we found 65 SNPs in CYP2C9 among which 1425A>T SNP was the most frequent (29 people [18%]). VKORC1 SNP 1639G>A was not detected in our population. 67% of respondents had 174–136C>T SNP with might be associated with warfarin response. 55 (34.8%), 37 (23.4%) and 45 (28.5%) people had 173 + 324T>G, 283 + 124G>C and 367 + 308T>C SNPs correspondingly.

Conclusions: Our study clearly shows that more than 30% of patients might experience side effects after warfarin therapy without prior genetic testing due to high frequency of CYP2C9*2 and CY2PC9*3 variants in population. At the same time VKORC1 impact of most frequent SNPs needs further investigation.

Acknowledgements: The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

P114-T | In-hospital acute coronary syndrome management in Europe: efficacy-proven pharmacological treatment trends 2000–2010, and its impact on vital status at discharge

I. Dégano*,†; S.H.Y. Kai‡; I. Kirchberger§,¶; D. Farmakis**; L. Tavazzi††; J. Marrugat*,†; J. Ferrieres‡‡

*CIBERCV, Instituto de Salud Carlos III, Madrid, Spain; †REGICOR Group, Hospital Del Mar Medical Research Institute (IMIM), Barcelona, Spain; ‡Department of Epidemiology, Health Economics and Public Health, Toulouse University Hospital, Toulouse, France; §MONICA/KORA Myocardial Infarction Registry, Augsburg, Germany; ¶Helmholtz Zentrum München, German Research Center for Environmental Health, Institute for Epidemiology II, Neuherberg, Germany; **Heart Failure Unit, Department of Cardiology, Attikon University Hospital, Athens, Greece; ††Maria Cecilia Hospital, GVM Care & Research, E.S. Health Science Foundation, Cotignola, Italy; ‡‡Department of Cardiology, Toulouse Rangueil University Hospital, Toulouse University School of Medicine, Toulouse, France

Background: Acute coronary syndrome (ACS) early drug management can be decisive for patient in-hospital prognosis. Our aim was to describe the variations of in-hospital proven-efficacy pharmacological treatments of ACS patients in Europe from 2000 to 2010, and to assess the association between their use and vital status at discharge.

Materials and methods: The EUROTRACS (EUROpean Treatment & Reduction of Acute Coronary Syndromes cost analysis) database was used for the analysis. EUROTRACS contains data from European studies and surveys, and from national/regional clinical registries of ACS patients. Prescriptions of beta blockers, statins, angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB), and aspirin were considered. Descriptive analyses were performed for the whole sample, and separately for ACS patients with and without ST elevation (STEACS and non-STEACS, respectively). A backward multivariate logistic regression model was used to evaluate the association between vital status at discharge and treatment prescriptions.

Results: Due to missing data regarding the variables of interest (>20%), only data from the Euro Heart Surveys I and II, the Greek, German and Spanish registries (HELIOS, MONICA/KORA, and MASCARA, respectively) were used. A total of 25 796 ACS cases were included, among them 45% were STEACS. Aspirin appeared to have been steadily prescribed (91% in 2000 and 98% of ACS patients treated in 2010), while there was an increase in the prescriptions of beta blockers (79–98%), of statins (50–94%) and of ACEI/ARB (60–92%). Similar trends were observed in STEACS and in non-STEACS patients. Use of the aforementioned drugs was associated with higher odds of survival at discharge in multivariate models.

Conclusions: Use of proven-efficacy pharmacological treatment in ACS patients improved from 2000 to 2010, and was associated to a better outcome at discharge.

P115-T | Influence of pathology of auditory analyzer on cardiac output

T.L. Zefirov; A.M. Golovachev; R.G. Biktemirova; A. Ibragimov; N.I. Ziyatdinova

Kazan (volga Region) Federal University, Kazan, Russian Federation

Congenital disorders of the auditory analyzer can influence the functioning of other physiological systems. The cardiac output of young people with disorders of hearing was studied in comparison with healthy people similar parameters.

The cardiac output from the left ventricle into the aorta was measured using Ultrasound Cardiac Output Monitor (USCOM, Australia) in young people 20–25 years old with disabilities having a pathology of hearing. The first group (gr.1) included young people completely devoid of hearing and with IV degree of hearing loss. The second group (gr.2) included young people with disorders of the auditory analyzer having I-III degrees of hearing loss. Healthy young people participated in the study as control group (gr.contr.) The AV (aortic valve) examination mode was selected on the monitor for aortic measure. The transducer was placed with the appropriate localization for this mode (suprasternal position). All the measurements were performed at rest.

Significant differences were revealed between the indicators of the minute distance (MD) ($P \leq 0{\cdot}05$), cardiac output (CO) ($P \leq 0{\cdot}05$) in gr.1 and gr.contr. These indicators were as follows: MD (Gr.1) – 26·71 ± 3·59 m/min; MD (Gr.2) – 16·58 ± 3·95 m/min; MD (Gr.contr.) – 17·33 ± 2·27 m/min; CO (Gr.1) – 7·81 ± 1·00 L/min; CO (Gr.2) – 5·36 ± 1·38 L/min; CO (Gr.contr.) – 5·16 ± 0·52 L/min.

The obtained data may indicate the influence of pathological processes in the hearing organs on the normal development of the cardiovascular system.

Work supported by Program of Competitive Growth of KFU and Russian Foundation for Basic Research.

P116-T | Angiographic predictors of experimental infarct size in a swine model of reperfused myocardial infarction

V. Crisóstomo*,†; C. Baez-Diaz*,†; V. Blanco-Blazquez*,†; A. Abad-Cobo*; I. Gonzalez-Bueno*; J.A. Antequera-Barroso‡; J. Maestre*,†; F.M. Sanchez-Margallo*,†

**Centro De Cirugía De Mínima Invasión Jesús Usón, Caceres, Spain; †CIBER de Enfermedades Cardiovasculares, Madrid, Spain; ‡Mathematics and Experimental Sciences Department, University of Extremadura, Caceres, Spain*

Background: To realize the exciting potential of cardiac regenerative therapies rigorous translational models must be used. Swine infarct models are widely used for this purpose. However, the infarct sizes obtained via balloon occlusion of the porcine LAD are highly variable.

We set out to study the relationship between the anatomical features of the porcine LAD and the size of infarction (IS) measured with MRI at 1 week.

Material and methods: Twenty-four pigs surviving a 90 minutes balloon occlusion of the mid-LAD were used for this study. The following angiographic parameters were considered: Number of branches present, number of branches occluded, level of occlusion (expressed as percentage of arterial length occluded), presence of a distinct "ramus intermedius" and animal weight.

The relationship between these parameters and IS as measured by DE-MRI on day 7 after induction was studied using non parametric correlations and lineal regression. Moreover, an inclusion criterion of IS > 15% was defined and the capability of these variables to predict whether animals were going to meet it was determined using logistic binary regression.

Results: Significant correlations were found between IS and the number of branches present ($P = 0{\cdot}004$) and the number of branches occluded ($P = 0{\cdot}003$), while no correlation could be evidenced with the other parameters. However, while significant ($P = 0{\cdot}024$), lineal regression model could only predict 30% of observed IS. Logistic binary regression yielded a significant ($P = 0{\cdot}014$) model that could correctly predict 75% of cases, with 72·7% specificity and 76·9% sensitivity. While further work is needed to refine the model, with this tool a single angiographic procedure could be used to predict the probability of an experimental subject not meeting the inclusion criterion, thus allowing greater ethical refinement of the infarction procedure by decreasing the amount of animals used that will be discarded due to insufficient IS.

P117-T | Paramagnetic centers in atherosclerotic plaques of carotid arteries as indicators of their content and stability

K. Usachev*; G. Mamin*; M. Gafurov*; I. Ignat'ev†; R. Kamaltdinov†; M. Salakhov*,‡; F. Murzakhanov*; A. Kiiamov*; Y. Osin*; Y. Chelyshev*,§; S. Orlinskii*

*Kazan Federal University, Kazan, Russian Federation; †Interregional Clinical and Diagnostic Centre, Kazan, Russian Federation; ‡Tatarstan Academy of Sciences, Kazan, Russian Federation; §Kazan State Medical University, Kazan, Russian Federation

Background: Complex structural and functional changes occur in the arterial system with advancing age. Understanding the physical and chemical factors that are connected with the atherosclerotic plaque (ASP) calcification and stability is a matter of controversy and extensive investigations. Diversity of ASP, their complex structure and strong inhomogeneity require new research tools and approaches.

Material and methods: Plaque tissues of aorta walls from male patients with atherosclerosis were gathered postmortem or during carotid endarterectomy operations. The micromorphology and chemical content were defined by scanning electron microscopy (SEM, Merlin), electron dispersive spectrometer (EDS, AZtec), X-ray diffraction (XRD, D8-Advance). Pulsed electron paramagnetic resonance (EPR) s done at 94 GHz, T = 6–300 K (Bruker E-680). The results are compared with those obtained on synthetic calcium phosphates – hydroxyapatite (HA), tricalcium phosphate (TCP), octacalcium phosphate (OCP).

Results: Ca, P, O, Na, Mg, Cl, K, Cu elements were detected. From the SEM/EDS/XRD the presence of only HA in the calcified (Ca/P > 1·0) and other CaP (probably TCP and OCP) in samples with Ca/P < 0·6 was found. By XRD in some samples nanosized (<50 nm) HA deposits were detected. $Mn2^+$ and radiation induced CO_2- radicals were detected and identified by EPR. Their EPR spectral and relaxation characteristics depends on the calcification degree, location (cap, shoulders or core of ASP), ASP stability. Correlation ($P < 0{\cdot}05$) between the relaxation characteristics of $Mn2^+$ ions and ASP stability was found.

Conclusions: Due to the small sample volume (500 nL, 0·5 mm cross section) and high spectral resolution pulsed high-field EPR can be used as a tool to study different parts of ASP to follow the presence of native paramagnetic centers and calcification processes.

Acknowledgments: Study is done in the laboratories guided by the Program of Competitive Growth of KFU, supported by the RFBR (grant 18-415-160015).

P118-T | Involvement of large-conductance calcium-activated potassium channels in pinacidil effects on the isolated bypass grafts from patients with and without type-2 diabetes mellitus

J. Rajkovic*; M. Peric†; J. Stanisic‡; J. Rakocevic§; R. Novakovic*; V. Djokic*; M. Labudovic-Borovic§; S. Tepavcevic‡; H. Heinle¶; L. Gojkovic-Bukarica*

*Institute of Pharmacology, Clinical Pharmacology and Toxicology, Medical Faculty, University of Belgrade, Belgrade, Serbia; †Dedinje Cardiovascular Institute, Belgrade, Serbia; ‡Laboratory for Molecular Biology and Endocrinology, Vinca Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia; §Institute of Histology and Embryology, Medical Faculty, University of Belgrade, Belgrade, Serbia; ¶Institute of Physiology, University of Tuebingen, Tuebingen, Germany

Previously, we have shown that pinacidil, a potassium channel opener (PCO), induced potent endothelium-independent relaxation of bypass grafts obtained from patients who undergoing bypass graft surgery. Its mechanism of relaxation is partly correlated with an interaction with smooth muscle ATP-sensitive potassium (K_{ATP}) channels, but also included other types of K channels. Following this observation we conducted the study to investigate the contribution of large-conductance calcium-activated potassium (BK_{Ca}) channels in the pinacidil-induced relaxation of bypass grafts obtained from patients with/without type-2 diabetes mellitus (T2DM). Previously, it has been shown that expression of BK_{Ca} channels is lower in blood vessels obtained from diabetic animals.

Rings of human saphenous vein (HSV) and human internal mammary artery (HIMA), without endothelium, were mounted in an organ bath system and isometric tension was being recorded. Pinacidil (0·01–100 μM) was used for relaxation of HSV and HIMA precontracted with phenylephrine (0·1 mM) and 5-hydroxytryptamine (0·1 mM), respectively. The expression of BK_{Ca} subunit (MAXI-Kα) was detected by Western blot and by immunohistochemistry using segments of HSV and HIMA obtained from patients with/without T2DM.

Pinacidil produces concentration-dependent vasorelaxation of HSV and HIMA obtained from diabetic patients. Tetraethylammonium (1 mM), a nonselective blocker of K_{Ca} channels and iberiotoxin, a highly selective blocker of BK_{Ca} channels (0·1 μM) did not antagonize the effect of pinacidil on HSV and HIMA obtained from patients with/without T2DM ($P > 0{\cdot}05$). There was no differences in expression of MAXI-Kα subunit comparing the segments of grafts obtained from patients with/without T2DM.

Thus, BK_{Ca} channels are expressed in the vascular smooth muscle, but they are not involved in the dilatation of grafts induced by pinacidil. The expression of MAXI-Kα subunit on HSV and HIMA was equal in both group of patients and do not contribute to the differences between grafts obtained from patients with/without T2DM.

P119-T | Myocardial remodelling post-infarction: extracellular matrix regulation and the Complement C3-system

M. Garcia-Arguinzonis*,†; G. Vilahur*,†; E. Diaz*,†; L. Badimon*,†; T. Padro*,†

Program ICCC-Institut Català de Ciències Cardiovasculars, IR-Hospital de la Santa Creu i Sant Pau, UAB, Barcelona, Spain; †CiberCV, Institute Carlos III, Barcelona, Spain, Barcelona, España

Structural remodeling post-myocardial infarction (MI) is a major determinant of progression to heart failure. The matrisomal-fraction (functional non-structural proteins) of the extracellular matrix (ECM) has been related to cardiac remodeling post-MI, but remains poorly understood. The C3-complement system, as part of the ECM-matrisome, seems to play a relevant role in tissue remodeling. This study aimed to characterize the C3-system signature in the ECM of revascularized hearts.

Methods: The study was performed in the ECM-matrisome of the peri-infarct region (penumbra) of porcine myocardium. Myocardial infarction was induced by coronary balloon occlusion in closed chest normocholesterolemic pigs. After 1·5 hours of LDA occlusion the heart was revascularized for 2·5 hours, 1, 3 and 30 days and animals were euthanized (n = 7 pigs/time-point). The matrisomal-fraction was obtained by sequential protein extraction and solubilized in guanidin-buffer. Protein analysis was performed by western blot.

Results: The C3-system was consistently detected in the ECM-matrisome post-MI, with an evolving protein-signature in relation to the elapsed time post-revascularization (PostR) for C3 and the C3-system regulatory proteins CFHR5 and CFH. C3 and C3-products (derived of complement-system activation) were significantly increased in the penumbra-zone at 2·5 hours and 1 day PostR, but the increase was not seen at longer periods PostR, indicating an acute C3-system response to the myocardial damage. Accordingly, CFHR5 (a positive regulator of the C3-cascade) was 4-fold decreased ($P < 0·01$) at day 1, remaining low after 30-day PostR, and CFH (C3-cascade activation inhibitor), showed the opposite pattern, reaching the highest values at 30-day PostR.

Conclusion: Our results demonstrated a timely coordinated response of the C3-System in the myocardium as a response to infarction and revascularization up to 30 days postintervention. In-depth knowledge of the ECM-matrisomal composition in the ischemic myocardium is needed to timely intervene and better define novel cardioprotective strategies.

P120-T | HSP expression in the subcutaneous and omental adipose tissue of diabetic and obese patients

C. Lambert*,†; L. Badimon*,†

Program ICCC-Institut Català de Ciències Cardiovasculars, IR-Hospital de la Santa Creu i Sant Pau, UAB, Barcelona, Spain; †CiberCV, Institute Carlos III, Barcelona, Spain

Background: Diabetes mellitus (DM) and obesity (OB) are highly prevalent risk factors for cardiovascular disease, commonly associated with chronic subclinical inflammation and increased oxidative stress. Heat shock proteins (HSP) are highly conserved chaperones involved in the maintenance of proteins in their functional native conformation. The metabolic disturbances occurring during DM and OB induce cellular stress and we have hypothesized that they can influence HSP-regulation and hence cellular homeostasis. Here we have study HSP-regulation in the adipose tissue (AT) of OB and DM patients.

Methods: Subcutaneous and Omental human-AT were obtained during bariatric surgery and classified in 3 groups (N = 6 each); (i) type-III obese (OBIII) diabetic patients; (ii) OBIII non-diabetic patients; (iii) type-I obese (OBI) diabetic patients. ATs were collected in lysis buffer and processed for proteomic analysis. Samples were analyzed by two-dimensional gel electrophoresis and mass-spectrometry. The mitochondrial HSP60 and the anti-inflammatory and antiapoptotic HSP70-family and HSP27 proteins were identified and analyzed.

Results: DM and non-DM patients have different levels of HSP in omental and subcutaneous-AT. Levels of HSP27 were lower in both types of WAT in DM ($P < 0·05$). On the contrary the levels of HSP70/71 were increased in subcutaneous-AT in DM ($P < 0·03$). Levels of HSP60 and GRP78BiP were unchanged. In obese non-diabetic patients there was no difference in protein levels (HSP27, HSP70-family and HSP60) in omental and subcutaneous-AT obtained from the same patient. Instead within diabetic patients, HSP60 was found at higher levels in the omental-AT than in the subcutaneous-AT ($P = 0·04$) in agreement with the higher metabolic distress and oxidation found in visceral-fat. A higher protein content was found in subcutaneous-AT for HSP70 ($P = 0·007$) and no change for HSP27.

Conclusions: Our results indicate that the presence of diabetes induces higher cellular stress than the presence of obesity. HSP are differentially regulated to ensure cell function under the higher metabolic stress. Further studies are needed to clear the meaning of the changes in different HSP regarding cell function.

P121-T | Immunohistological detection of FBN1 expression in mouse aorta

L.S. Lsicanelas; M.J.R. Rodriguez; M.R.C. Garcia; J.M. Jmredondo

Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain

Marfan syndrome (MFS) is a systemic hereditable autosomal dominant disease characterized by connective tissue disorders. Acute aortic syndrome (AAS) including aortic dissection is the cause of death in over 90% of untreated patients. Fibrillin 1 (FBN1) has been identified as the major causing gene of MFS, as nearly 3000 FBN1 mutations has been associated with Marfan disease, but rarely with dissection events. FBN1 is an important structural protein which regulates microfibril stability and assembly. FBN1 mutations disrupt microfibril formation, and eventually weaken the connective tissue. Therefore, dysregulation in FBN1 content could play an important role in aneurysm formation. Histopathology of aneurism is characterized by an enlargement and weakened aortic medial layer, with fibrosis and disorganization and fragmentation of the elastic fibers.

We have used two different models of Marfan mice to analyze in aorta gene expression changes in Fbn1.

1. A mouse heterozygous for an allele of Fbn1 (Fbn1C1039G/+) containing a mutation, frequently found in MFS patients.
2. A mouse model based on lentiviral-mediated Fbn1 silencing.

Our results indicate that lentivirus encoding Fbn1 specific shRNA efficiently downregulated aortic Fbn1, leading aortic dilation and medial degeneration. Given the difficulties to detect Fbn1 expression in mouse aortic extracts by Western Blot, we have set up an immunohistological protocol to detect Fbn1 based on digestion of the aortic tissue with elastase. This approach has allowed us to monitor the levels of Fbn1 protein expression in wild type, in Fbn1-deficient mice, and compare them Fbn1 expression in Marfan patients.

P122-T | The influence of methoxamine on the isolated heart chronotropy and inotropy

T.L. Zefirov*; I.I. Khabibrakhmanov*; J.T. Zefirova†; N.I. Ziyatdinova*

**Kazan (volga Region) Federal University, Kazan, Russian Federation; †Department of Internal Medicine, Saint Mary's Hospital, Waterbury, USA*

It had earlier been shown that the combined blockade of β1-, β2-, and α1-ARs is more effective than the selective blockade of β-ARs, which is widely used in the treatment of cardiac pathologies. α1-AR of the heart participates in numerous physiologic processes, such as inotropy, genes transcription, protein synthesis, glucose metabolism and inhibition of apoptosis. These findings attest to the need of further studies to develop novel approaches in the treatment of cardiac pathologies.

The experiments were carried out on albino rats aged 20 weeks. The rats were anesthetized intraperitoneally with 25% urethane (800 mg/kg body weight). The heart was perfused in a Langendorff System (ADInstruments) with carbogen-oxygenated Krebs–Henseleit solution ex vivo. The retrograde perfusion was driven by constant hydrostatic pressure of 60–65 mmHg. To stimulate α1-ARs, methoxamine (MX, an agonist affecting all subtypes of α1-ARs, Sigma) was used at the concentrations of 10^{-10}–10^{-8} M. The signals were recorded in a PowerLab 8/35 system (ADInstruments) with the help of LabChart Pro 8.0 software. The data was processed statistically using Microsoft Excel software and Student's *t* test.

The stimulation of α1-ARs with MX led to bradycardia in the isolated heart. It was also observed that all studied concentrations of methoxamine induced a negative inotropic reaction of the isolated left ventricle of rats. The intensity of the negative inotropic effect depended on concentration of the agonist. Decreased heart rate and myocardium contractility with the activation of α1-AR may be secondary to decreased ICa via the activation of protein kinase C. It is quite possible that α1-AR participates in more delicate regulations of cardiac function and it is most likely that the effects of this stimulation depends on activities of other receptors and different intracellular systems.

Work supported by Program of Competitive Growth of KFU and Russian Foundation for Basic Research.

P123-T | The influence of If inhibition on the myocardium electrical activity

T.L. Zefirov*; L.I. Faskhutdinov*; A.L. Zefirov†; N.I. Ziyatdinova*

**Kazan (volga Region) Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation*

"Funny currents" (If) play a decisive role in the creation of automatic activities in the cells of mammalian pacemakers. Recently, new data were obtained indicating a possible involvement of If in the performance of working cardiomyocytes. This work was designed to study changes in the shape of the action potential (AP) of rat atrial cardiomyocytes induced by a specific inhibition of If with 10^{-5} M

of ZD7288 (an organic blocker) stimulated electrically and also in the absence of this stimulation.

The experiments, which involved the intracellular recording of electrical activities in the working myocardium, were carried out on random-bred albino rats. Isolated right atrial wall from a fragment of the right auricle exhibiting no pacemaker activity was placed in a 3-mL chamber and superfused with Tyrode solution at 38°C at a rate of 10 mL/min. The stimulus duration (1 ms) and repetition rate (5 Hz) corresponded to the normal HR of mature rats. Intracellular AP was recorded via glass microelectrodes with resistance of 25–60 MΩ. The signals were digitized with an E14-140 converter (L-Card) and recorded using PowerGraph 3.3 software (DiSoft). The data were processed with Mini-Analysis 3.0.1 software (Synaptosoft), Microsoft Excel software and Student's *t* test.

ZD7288 significantly increased the duration of action potentials at 50% and 90% repolarization levels in atrial myocardium at a fixed stimulation rate of 5 Hz. The blocker affected neither resting potential nor the upstroke velocity of action potential.

Work supported by Program of Competitive Growth of KFU and Russian Foundation for Basic Research (grant No. 17-04-00071).

P124-T | The blockade of If in isolated (Langendorff perfused) heart

T.L. Zefirov*; A.M. Kuptsova*; T.P. Zefirova†; L.I. Faskhutdinov*; N.I. Ziyatdinova*

**Kazan (volga Region) Federal University, Kazan, Russian Federation; †Kazan State Medical Academy – Branch Campus of the FSBEI FPE RMACPE Russia, Kazan, Russian Federation*

According to modern views, If are responsible for the development of initial, linear, and slow diastolic depolarization in cells of the sinoatrial node. At the same time, a rather small If was identified in both atypical and working cardiomyocytes. Therefore, it remains unclear how the blockade of data currents affects heart function.

This research aim is to investigate dose-dependent effects of the blockade of If: on coronary flow; the inotropy; and the chronotropy in Langendorff perfused heart in adult rats. Isolated hearts were perfused in a Krebs-Henseleit solution – Langendorff (ADinstruments) installation. The coronary flow (CF), systolic pressure in the left ventricle (LVP) and heart rate (HR) were calculated along the curve. The signals were recorded in a PowerLab system (ADInstruments) with the help of LabChart Pro 8.0 software. 10^{-9}–3×10^{-5} M concentrations range of ZD7288 (Sigma) were used for the blockade of If. The data was processed statistically using Microsoft Excel software and Student's *t* test.

ZD7288 10^{-9} M increased LVP by 47% ($P \leq 0{\cdot}05$), decreased HR by 26% ($P \leq 0{\cdot}05$) and reduced CF by 20% ($P \leq 0{\cdot}01$). ZD7288 10^{-8} M, 10^{-7} M and 10^{-5} M did not cause significant alterations in the studied parameters of the heart. ZD7288 10^{-6} M led to bradycardia – 23% ($P \leq 0{\cdot}05$) and did not cause significant changes in LVP and CF. ZD7288 3×10^{-6} M reduced LVP by 14% ($P \leq 0{\cdot}05$), HR by 11% ($P \leq 0{\cdot}05$) and did not lead to a change in CF. If blockade 3×10^{-5} M reduced myocardial inotropy by 26% ($P \leq 0{\cdot}05$), CF by 14% ($P \leq 0{\cdot}01$) and HR by 19% ($P \leq 0{\cdot}05$).

The blockade of If in Langendorff perfused hearts of adult rats resulted in different contractility effects. The range in all the studied concentrations of the If blockade reduced both heart function and coronary flow.

Work supported by Program of Competitive Growth of KFU and Russian Foundation for Basic Research (grant No. 17-04-00071).

P125-T | Role of NPY1,5-receptors in the neonatal rats myocardial contractility

T. Zefirov*; A.A. Zverev*; N. Iskakov*; P.M. Masliukov†; T.A. Anikina*

**Kazan (volga Region) Federal University, Kazan, Russian Federation; †Yaroslavl State Medical University, Yaroslavl, Russian Federation*

Neuropeptide Y (NPY) is present in the central and peripheral nervous systems and fully satisfies to neurotransmitter criteria, since it is stored in sympathetic vesicles, released by electrical stimulation and acts on specific receptors. In the rat heart there are metabotropic Y1R, Y2R, Y3R, Y4R and Y5R receptors. The density of different receptor subtypes varies in postnatal ontogenesis. Expression of Y1R increased between 10 and 20 days of life. A small number of Y2R is observed in the atria and ventricles only from 20 days of life. In contrast, the highest level of expression of Y5R was found in newborn pups comparing with more adult rats.

The aim of the current study was to determine the role of different subtypes of NPY receptors in the heart contraction in the postnatal development. Registration of isometric contraction of atrial and ventricular myocardial striae of 7- and 100-day-old rats was carried out on a PowerLab device with a force sensor MLT 050/D (ADInstruments).

The selective agonist of Y1R, Leu(31)Pro(34)NPY (10^{-5}–10^{-13} M), induced an increase in myocardial contraction force in 7-day-old (10^{-6} M) and in 100-day-old rats (10^{-7} M). The selective blocker of Y1R, BIBP 3226,

eliminates the positive effect caused by Leu(31)Pro(34) NPY in all age groups, which indicates the involvement of this receptor subtype in myocardial contractility.

NPY (10^{-6}–10^{-10} M) reduced the force of myocardial contraction in 7-day-old animals and does not cause significant changes in the parameters of isometric myocardial contraction in 100-day-old rats. NPY in the presence of selective blocker of Y5R, CGP 71683 (1·4 mM), reduced the force of myocardial contraction in 7-day-old animals and did not affect in 100-day-old animals, which indicates the involvement of this receptor subtype in myocardial contractility only in newborn animals.

Work supported by Program of Competitive Growth of KFU and Russian Foundation for Basic Research (grant No. 17-04-00071).

P126-T | Developmental changes of ATP influence to rats heart parameters

T.L. Zefirov*; A.A. Zverev*; T.A. Anikina*; N. Iskakov*; P.M. Masliukov†; A.L. Zefirov‡

*Kazan (volga Region) Federal University, Kazan, Russian Federation; †Yaroslavl State Medical University, Yaroslavl, Russian Federation; ‡Kazan State Medical University, Kazan, Russian Federation

ATP can participate in the intercellular signaling, where it acts as a cotransmitter on specific purinoreceptors. In the rat cardiomyocytes have been found ionotropic P2X1,2,4,5 and metabotropic P2Y1,2,4,6,11 receptors. The results of the studies are contradictory, because ATP rapidly dissociates to adenosine, which acts on its receptors causing a multidirectional effect. During the postnatal development, the percentage of P2X2 and P2X6 is kept at the same level with a peak for P2X3 purinoreceptors at 20 days.

The influence of ATP on the heart has been studied. Registration of isometric contraction of atrial myocardium strips was performed with a preserved sinus node and stimulation of 6 pulses per minute in 7- and 100-day-old rats. Intracellular recording of the electrical activity was performed using glass microelectrodes with a resistance of 30–60 MΩ.

ATP with a concentration of 10^{-4}–10^{-7} M causes a dose-dependent reduction in the striae of the myocardium of the atria and ventricles. The maximum increase was observed in the concentration of 10^{-7} M in newborns and 10^{-6} M in adult animals. When ATP was added to the strips of the myocardium with a preserved sinus node, a short-term increase in the frequency and force of contraction results was found. Increasing the concentration of the agonist led to a decrease in the strength of contraction of the myocardium strips. Adding this concentration to the atrial preparation with a preserved sinus node caused a short-term increase in the heart rate, an increase in the duration of 20%, 50%, and 90% of the repolarization.

The increase of myocardial contractility with the addition of ATP is associated with the activation of P2X1 purinoreceptors which play the most important role in the positive inotropic effect in newborn rats.

Work supported by Program of Competitive Growth of KFU and Russian Foundation for Basic Research (grant No. 17-04-00071).

P127-T | Involvement of α-adrenoreceptors of rats myocardial contractility dopaminergic regulation during postnatal ontogenesis

T. Zefirov; G.A. Bilalova; F.G. Sitdikov; N.B. Dikopolskaya; M.V. Shakhelislamova

Kazan (volga Region) Federal University, Kazan, Russian Federation

The regulatory effect of dopamine, especially in ontogenesis, on myocardial contractility has been given little attention. The function of dopamine is initiated through the activation of dopamine receptors found in the heart of the rat and human. Dopamine also interacts with α- and β-adrenergic receptors. The purpose of this study is to study the effect of different dopamine concentrations on myocardium contractility in 42-, 56- and 100-day-old rats with blockaded α-adrenergic receptors.

Registration of isometric contraction of atrial and ventricular myocardial striae of 42-, 56- and 100-day-old rats was carried out on a PowerLab device with MLT 050/D force sensor (ADInstruments). We determined the reaction contraction force of the atrium and ventricle myocardium at dopamine range of 10^{-5}–10^{-9} M. 10^{-6} M concentration of phentolamine was used for the blockade of α-adrenergic.

Dopamine blockade by phentolamine increased the force of atrial contractions by 8% (10^{-6} M) and in the ventricles by 15% (10^{-5} M) in 42-day-old rats after. All the other dopamine concentrations lead to a decrease in contractility of strips of myocardium of Atria and ventricles.

Phentolamine induced dopamine blockade increased the force of contraction of the Atria and ventricles by 13–20% (10^{-5}, 10^{-6}, 10^{-9} M) in 56-day-old rats. We observed a 19% reduced contraction force of the atrial and ventricular strips of the myocardium after treatment with 10^{-7} and 10^{-8} M concentrations of dopamine.

100-day-old animals, phentolamine induced dopamine blockade increased the force of atrial contractions in the studied range of concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-9} M) and reduces the force of contraction of strips of

ventricular myocardium. Consequently, dopamine and α-adrenergic receptors are not responsible for the decrease in strength of contractility of the ventricular myocardium.

Work supported by Program of Competitive Growth of KFU and Russian Foundation for Basic Research.

P128-T | Endovascular revascularization of occlusion renal artery in a patient with chronic kidney disease

S. Zinchenko*; A. Khalirakhmanov*; A. Sharafeev*; J. Troshina†

*Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation

Background: The problem of revascularization of renal arteries in chronic kidney disease remains unsolved. We present the case of endovascular revascularization of renal arteries with positive dynamics and effect.

Materials and methods: Revealing renal arteries stenosis and performed endovascular stenting with the evaluation of the function in 6 months.

Results and discussion: Patient M, 57 years old, entered to the hospital with weakness, dizziness, arterial hypertension. Glomerular filtration rate (GFR) was 28 mL/min/1.73 m^2 (CKD-EPI). The daily blood pressure was 185/110 mmHg. Complete occlusion of the right renal artery and the proximal segment of the left renal artery to 85% were revealed by Doppler ultrasonography. Angiography of renal arteries with stenting occlusion of the right renal artery was performed. Stent in the left artery was emplaced in 3 months.

In 6 months after these procedures patient reported improvement in his general condition, blood pressure fixed at 140/80 mmHg. We received stabilization of renal function: serum creatinine concentration of the blood dropped to 67·8 mmol/L, GFR increased from baseline – 86 mL/min/1·73 m^2. The average systolic blood pressure fell by 32·1%, diastolic blood pressure decreased by 27·3% from baseline (75 mmHg). According to dynamic nephroscintigraphy, stabilization of renal function was with preservation of the perfusion volume of the right kidney, without significant increase in function, with improvement of left kidney function. The total GFR was 55·56 mL/min (left – 46·5 mL/min, right – 9.06 mL/min).

Conclusion: This case shows that percutaneous endovascular interventions can be effective and safe as a method of revascularization in patients with renal artery occlusions.

Work supported by Program of Competitive Growth of KFU.

P035-F | Ventricular or atrial epicardial fat secretome can be regulated by acetylcholine: new preclinical models on autonomic dysfunction

M. Couselo-Seijas*; R.M. Agra*,†,‡; A.L. Fernández§; J.M. Martínez-Cereijo§; S.B. Bravo¶; J.N. López-Canoa*; J.L. Martínez-Sández*,†,‡; J. García-Seara*,†,‡; J.R. González-Juanatey*,†,‡; M. Rodríguez-Mañero*,†,‡; S. Eiras*,†,‡

*Cardiology Group, Health Research Institute of Santiago de Compostela, Santiago De Compostela, Spain; †Cardiovascular Area and Coronary Unit, University Clinical Hospital, Santiago de Compostela, Spain; ‡CIBERCV, Madrid, Spain; §Heart Surgery Department, University Clinical Hospital, Santiago de Compostela, Spain; ¶Proteomic Unit, Health Research Institute of Santiago de Compostela, Santiago de Compostela, Spain

Background: Atrial fibrillation (AF) is one of the most sustained arrhythmias. Recent studies suggest that the local amount of epicardial adipose tissue (EAT) around the atria is more associated with AF burden than obesity, ageing or left atria dimensions. Several authors have paid attention on EAT-released proteins as mediators of AF substrate. Besides, EAT shelters ganglionated plexuses and cholinergic and adrenergic nerves. The principal cholinergic neurotransmitter is acetylcholine (ACh), which acts reducing the action potential duration. Some authors select EATv for studying EAT contribution to AF while others suggest the knowledge of the adipose tissue closer to the disorder.

Purpose: We wanted to compare the secretome between peri-atrial EAT (EATa) and peri-ventricular EAT (EATv) and its differential regulation by acetylcholine (ACh).

Material and methods: EATa and EATv from 11 patients underwent open heart surgery were splitted in 100 mg pieces and cultured. After 24 hours washing, tissue proteins were separated by 2-Dimensional electrophoresis. Secretome proteins were separated by SDS-polyacrylamide electrophoresis gel, quantified by an analysis software and identified by mass spectrometry. Muscarinic receptor type's expression was analyzed by real time polymerase chain reaction or western blot. Then ACh and acetylcholinesterase (AChE) activities were determined by colorimetric assays.

Results: Our results showed high similarities between EATa and EATv regarding to their protein and secretome profiles. Thus, 282 common proteins were identified in both tissues. EATa and EATv contained muscarinic receptor type 3 (mAChR 3), which is increased in adipogenesis-induced cells. Despite AChE activity was higher in EATa (128 [17–543 mU/mg tissue]) than in EATv (43 [8–142 mU/mg tissue]); $P < 0{\cdot}05$, both tissues modified their released protein profile after ACh treatment.

Conclusions: The similarity between the released proteins from EATa and EATv and its regulation by ACh makes them an appropriate preclinical model to clarify the interplay among EAT, AF and autonomic dysfunction.

P036-F | Protective effect of lipoxin A_4 analog BML-111 on a murine model of autoimmune myocarditis

R.I. Jaén*,†; A. Povo-Retana*,†; V. Terrón*; A. Val-Blasco‡; M. Fernández-Velasco†,‡; L. Boscá*,†; P. Prieto*,†

**Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), Madrid, Spain; †Instituto de Investigación Sanitaria del Hospital Universitario La Paz (IdiPAZ), Madrid, Spain; ‡Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain*

Myocarditis is a cardiovascular disease characterized by a chronic inflammation of the myocardium that causes cardiac dysfunction. In the majority of patients this disease leads to dilated cardiomyopathy and represents the major cause of sudden cardiac death in young adults. Although it is a clinically severe disease, current treatments are inefficient and unspecific. In search for new therapies, lipoxins and their derivatives arise as a more effective and safer alternative thanks to their pro-resolving properties. The present work aimed to evaluate the effect of BML-111, a lipoxin A_4 synthetic analog, in a murine model of experimental autoimmune myocarditis (EAM) through molecular, histological and echocardiographic studies. In this EAM model, mice were immunized to cardiac myosin at day 0 and 7 of the experiment and daily treated with BML-111 from day 7 to 21. We discovered that BML-111 treatment significantly improved cardiac function and reduced cardiac hypertrophy of myocarditis-induced mice. Furthermore, BML-111 also diminished infiltration of inflammatory cells in the heart and prevented the fibrotic process associated to adverse cardiac remodeling. Finally, we also observed that the levels of pro-inflammatory and pro-fibrotic molecular mediators (TGFβ, IL-6, TNFα and Galectin-3) in the heart decreased upon treatment. Together, these results demonstrate that BML-111 mitigates cardiac alterations associated to myocarditis by restoring cardiac function and reducing adverse cardiac remodeling. These findings highlight the therapeutic potential of lipoxins to treat myocarditis and provide new insights on the development of future therapies to alleviate the outcome of inflammatory heart diseases.

P037-F | Hydrogen sulfide amplifies the negative inotropic effect of acetylcholine in mouse atrium

A. Blokhina; N. Khaertdinov; K. Chernova; G. Sitdikova

Kazan (volga Region) Federal University, Kazan, Russian Federation

Hydrogen sulfide (H_2S) is synthesized endogenously and has negative inotropic effects in cardiomyocytes and induces cardioprotection. The known targets of H_2S are ATP-dependent K+-channels (K(ATP)-channels), voltage-gated L-type Ca^{2+}-channels, protein kinase C, nitric oxide (NO) metabolism and phosphodiesterases. H_2S enhances the vasodilatory action of acetylcholine (ACh) in aortic rings. In heart, activation of muscarinic acetylcholine receptor (m-AChR) results in inhibition of adenylate cyclase, increase of potassium current and production of NO, which in turn regulates the activity of soluble guanylyl cyclase. m-AChR are involved in the mechanisms of pre- and post-conditioning by the opening of mitochondrial K(ATP)-channels. The aim of our study was to investigate mechanisms of interaction of H_2S and m-AChR in the regulation of atrial myocardial contractility using the methods of tensometry and Ca^{2+} transient in isolated mouse atria. Sodium hydrogen sulfide (NaHS, 300 μM) used as the donor of H_2S, had a negative inotropic effect and decreased the amplitude of Ca^{2+} transients in mouse atrium. An agonist of m-AChR (carbachol, 1 μM) reduced contraction force of atrium and did not change the negative inotropic effect of NaHS. At the same time, a preliminary incubation of atrium with NaHS significantly amplified the effects of carbachol on the atrial contractility. The inhibition of adenylate cyclase (MDL 12·330, 3 μM), guanylyl cyclase (ODQ, 10 μM) and NO-synthesis (L-NAME, 200 μM) has no any effects on H_2S-induced increase of negative inotropic effect of carbachol. At the same time glibenclamide (50 μM), an inhibitor of K(ATP)-channels, significantly attenuated the effects of NaHS on carbachol-induced decrease of atrium contractions. It was concluded that activation of K(ATP)-channels underlies the enhancement of the negative inotropic effect of m-AChR activation by H_2S in atrium cardiomyocytes. Work supported by Program of Competitive Growth of KFU.

P038-F | ApoA-1 oxidation in abdominal aortic aneurysm

E. Camafeita*; D. Martínez-López†; R. Roldán-Montero†; I. Jorge*; E. Burillo†; L.M. Blanco-Colio†; J. Egido†; J.-B. Michel‡; J. Vázquez*; J.L. Martín-Ventura†

**Laboratorio de Proteómica Cardiovascular, CNIC and CIBERCV, Madrid, Spain; †Laboratorio de Patología Vascular, FIIS-FJD-Universidad Autónoma and CIBERCV, Madrid, Spain; ‡INSERM U1148, Paris, France*

Background: High density lipoprotein (HDL) cholesterol levels are negatively associated with human abdominal aortic aneurysm (AAA), where arterial wall weakening results in permanent aortic dilation. When transported through the arterial wall ApoA1, the main protein component of HDL,

is prone to oxidative modifications that influence the vasculoprotective properties of the particle. We have measured the oxidation level of ApoA1 in AAA patients and investigated its relation with HDL functionality.

Material and methods: The oxidation of tryptophan 108 (Trp108) from ApoA1 was measured by a parallel reaction monitoring mass spectrometric assay. ApoA1 and malondialdehyde (MDA) expression in tissues was performed by immunohistochemistry. Proinflammatory cytokine expression (IL-1β) was analyzed by Q-PCR and ELISA.

Results: Increased oxidation of ApoA1 Trp108 as compared to healthy subjects ($n = 40$) was found in plasma samples from AAA patients. In addition, ApoA1 Trp108 oxidation was also increased in HDL isolated from homogenates of human AAA thrombus, where ApoA1 colocalized with MDA, with respect to healthy aortas. Furthermore, upon incubation of HDL with AAA-thrombus-conditioned media (HDL-T) ApoA1 Trp108 is oxidized compared to control HDL (HDL-C). Finally, incubation of endothelial cells with HDL-T for 24 hours induced an increase in IL-1β mRNA and secreted protein levels with respect to HDL-C.

Conclusions: The oxidative modification of ApoA1 is clearly associated with HDL dysfunctionality in AAA, suggesting a mechanistic link among oxidative stress, inflammation and diminished vasculoprotective function of HDL in AAA.

P039-F | Lung ultrasound may reduce heart failure hospitalizations: preliminary results from the LUS-HF trial

M. Rivas-Lasarte; J.F. Martinez; A.M. Benedicto; E.S. Gonzalez; J. Álvarez-García; L.L. Lopez; S.M. Pérez; V. Brossa; A. Sionis; E.R. Minguell; J.C. Cuscullola

Cardiology Department Hospital De La Santa Creu I Sant Pau, Barcelona, Spain

Background: Pulmonary congestion is expressed in the form of B-lines detected by lung ultrasound (LUS), which has proven to be a potent prognostic predictor of hospitalization and mortality in HF. However, it is still unknown if a treatment strategy guided by LUS in HF patients may improve outcomes.

Purpose: The objective of our study is to analyze whether a treatment guided by LUS in patients with HF reduces the combined endpoint of readmission for HF worsening or death in a 6-month follow-up.

Methods: LUS-HF (NCT02959372 at ClinicalTrials.gov) is a randomized, single center, simple blind clinical trial that enrolls patients older than 18 years who have been hospitalized for HF. The exclusion criteria are life expectancy less than 6 months or severe lung disease. Eligible patients are randomized into either the "LUS group" or the "control group." The follow-up consists of visits in the HF clinic at periods of 15 days, 1, 3, and 6 months after discharge. Both groups are examined with LUS, but the result of the test is only provided to the treating physician in the "LUS group."

Results: Clinical characteristics of the first 52 patients included were comparable in both groups. The primary endpoint occurred in the 42·3% of the "control group" vs 19·2% of the "LUS group" (log-rank test 0·077, figure).

Conclusions: According to the preliminary results of the LUS-HF trial, LUS guided treatment may reduce readmissions in HF patients.

P040-F | Involvement of Fibroblast growth factor-21 in alcohol-induced cardiomyopathy

G. Ferrer-Curriu*; M. Guitart-Mampel†; G. Garrabou†; F. Villarroya*; J. Fernández-Solà‡; A. Planavila*

**Biochemistry and Molecular Biomedicine Department, Faculty of Biology – University of Barcelona and CIBERON, Barcelona, Spain; †Muscle Research and Mitochondrial Function Laboratory, Cellex-IDIBAPS, Faculty of Medicine and Health Science – University of Barcelona, Internal Medicine Service – Hospital Clinic of Barcelona and CIBERER, Barcelona, Spain; ‡Alcohol Unit, Department of Medicine – Hospital Clinic University of Barcelona, Barcelona, Spain*

Background: Alcoholic cardiomyopathy (ACM) resulting from chronic alcohol consumption is one of the main contributors leading to heart failure. ACM is associated with histological, cellular and structural changes within the myocardium. We previously showed that Fibroblast growth factor 21 (FGF21) protects against cardiac hypertrophy acting in an endocrine/autocrine manner. We aimed to study the role of FGF21 in the cardiac damage induced by chronic alcohol consumption.

Material and methods: FGF21 gene expression levels and circulating levels were analyzed in human heart samples and blood samples from healthy donors or chronic alcoholic patients. Two month-old wild type (WT) and FGF21 knockout (FGF21-KO) mice were fed a 4% alcohol liquid diet or a calorie-adjusted control liquid diet for 12 weeks.

Results: We found that chronic alcohol patients present higher FGF21 expression levels in heart biopsies and increased FGF21 circulating levels. Our mice model of chronic alcohol consumption recapitulates the results in humans: higher FGF21 expression levels in heart and a tendency to increase the FGF21 circulating levels after chronic

alcohol intake. Furthermore, we found an increased heart weight/tibia length (HW/TL) ratio after chronic alcohol consumption indicating cardiac hypertrophy but we did not observed differences due to genotype. However, echocardiographic measurements showed that alcohol consumption significantly increased both aortic peak and E peak only in FGF21-KO mice indicating enhanced cardiac dysfunction (systolic and diastolic) when mice lacked FGF21. Moreover, we observed a marked induction of the cardiac hypertrophy marker gene atrial natriuretic factor (ANF) and the fibrosis-related gene Collagen-3 (Col3) in FGF21-KO mice after alcohol consumption compared to WT mice. Finally, Trichrome Masson staining confirmed that lack of FGF21 aggravates cardiac fibrosis after alcohol consumption.

Conclusions: Our results show that FGF21 expression is induced by chronic alcohol consumption. In addition, the lack of FGF21 aggravates cardiac damage produced by ACM.

P041-F | High intensity focused ultrasound for treatment of peripheral artery disease

M.H.A. Groen*,†; N.C. Morsink*,†; C.E.V.B. Hazenberg‡; G.J. de Borst‡; A.J. Teske*; P.A. Doevendans*,§; R. van Es*

**Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands; †Technical Medicine, University of Twente, Enschede, The Netherlands; ‡Department of Vascular Surgery, University Medical Center Utrecht, Utrecht, The Netherlands; §Netherlands Heart Institute, Utrecht, The Netherlands*

Background: Current treatment of peripheral artery disease (PAD) often involves surgical procedures as angioplasty and stenting. These procedures are invasive and carry associated complications, while long-term effects are limited. High intensity focused ultrasound (HIFU) is a non-invasive technique to create sub-millimetre thermal lesions. Dual-Mode Ultrasound Arrays (DMUA) is a combined technique for both ultrasound image guidance and subsequent delivery of HIFU therapy. Ultrasound-guided HIFU might be a promising non-invasive alternative for treatment of PAD. The aim of this study was to investigate the acute effect of HIFU therapy on the femoral artery and the surrounding tissue in a porcine model with respect to safety.

Methods: In three pigs (50 kg), the diameter of the femoral artery and blood flow was measured before treatment, using diagnostic ultrasound and angiography. HIFU therapy was applied to the dorsal wall of the femoral artery using a 3·5 MHz, 64-element DMUA transducer, with intensities of 4000–5600 W/cm². A continuous 2·5–3·5 cm lesion was created by delivering 6–8 HIFU shots per imaging plane perpendicular to the artery, for 25–35 planes spaced 1 mm apart. After therapy, diameter and blood flow were measured, the skin was examined and the target area was removed for histological analysis using a tetrazolium test.

Results: No visible damage to the skin due to heating was observed. The artery showed acute spasms that persisted throughout the procedure. This effect gave a direct indication HIFU therapy was delivered at the correct location. Angiography showed a decreased flow and arterial diameter. Histology showed no signs of excessive damage to the surrounding tissue.

Conclusion: HIFU therapy was delivered without adverse effects besides arterial spasms. Spasm is thought to be transient, however further research is needed to study long-term effects.

P042-F | A simple CHA2DS2-VASc derived score for assessing long-term prognosis in STEMI patients (CardioCHUS STEMI registry)

A.R. Diéguez; B.C. Alvarez; R.T. Nouche; A.Á. Carrillo; F.G. Peña; X.S. Pena; B.Á. Álvarez; D.L. Otero; J.R.G. Juanatey

CHUS (Complexo Hospitalario Univeristario de Santiago de Compostela) CIBERCV, Santiago De Compostela, Spain

Aims: There are multiple clinical scores for assessing the prognosis in STEMI patients, nevertheless, most of these clinical scores are complex and time-consuming. The CHA2DS2-VASc score is a simple score developed for predicting thromboembolic events in patients with atrial fibrillation.

Our aim was to develop a user-friendly score, derived from the CHA2DS2-VASc score, for assessing the long-term outcomes of STEMI patients.

Methods and results: A cohort study was conducted including 1723 consecutive STEMI patients admitted to our hospital who underwent to pPCI between January 2008 and December 2016.

The C2HA2D2-VASC score was calculated using variables from the CHA2DS2-VASC score who were positively correlated with MACE in the univariate or multivariate analysis. Killip class was added to the score due to its powerful association with events and its ease implementation to the clinical practice. The C2HA2D2-VASC score included: heart failure(previous or Killip >1 at admission), age >75 years and diabetes mellitus which scored 2 points; hypertension, female sex and previous myocardial infarction or peripheral vascular disease which scored 1 point. Patients were classified as high-risk if they had a C2HA2D2-VASC score ≥2 points.

49·5 % of patients were classified as high risk by the C2HA2D2-VASC score. Low-risk patients were younger, more frequently on smoking, had a lower extension of coronary artery disease and lower GRACE score.

The C2HA2D2-VASC was positively correlated with all-cause death and MACE during follow-up (859 days).High-risk patients had a higher mortality (20% vs 2%, HR 8·274 [5·308–12·896], $P < 0·001$) and more MACE (30% vs 14%, HR 2·398 [1·932–2·976], $P < 0·001$) The C2HA2D2-VASC score showed a similar discriminative ability for predicting all-cause death and MACE showing no significative differences by comparison of ROC curves

Conclusions: The C2HA2D2-VASC score is positively correlated with worse long-term prognosis in our STEMI registry with a similar discriminative ability compared to the GRACE score.

P043-F | Six degrees of freedom robotic arm to guide high intensity focused ultrasound therapy for atherosclerotic plaques

N.C. Morsink*,†; M.H.A. Groen*,†; C.E.V.B. Hazenberg‡; G.J. de Borst‡; A.J. Teske*; P.A. Doevendans*,§; R. van Es*

**Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands; †Technical Medicine, University of Twente, Enschede, The Netherlands; ‡Department of Vascular Surgery, University Medical Center Utrecht, The Netherlands; §Netherlands Heart Institute, Utrecht, Netherlands*

Background: In the search for novel treatment options for atherosclerosis, high intensity focused ultrasound (HIFU) has shown promising results in terms of feasibility and acute safety. HIFU is non-invasive and can produce precisely located millimeter thermal lesions with little or no damage to surrounding tissue. To ensure continuous lesion formation within plaques, subsequent discrete HIFU shots need to be delivered for multiple transverse planes in direction of the target vessel. To evolve HIFU to a standard treatment modality for atherosclerosis, precise localization and targeting of atherosclerotic plaques is required. We have investigated the feasibility of using the robotic arm to guide HIFU therapy.

Methods: The HIFU transducer was attached to a six degrees-of-freedom UR3 robot (Universal Robots). The robot was linked to MATLAB (MathWorks) software to allow submillimeter movement in any direction. In three pigs (50 kg), 6–8 HIFU shots were delivered to the dorsal wall of the femoral artery, in 25–35 transverse planes spaced 1 mm apart. All 3D ablation positions were saved and visualized in real-time. The 3D distance to the previous ablation position was displayed to allow precise spacing between subsequent ablations and ensure a continuous lesion.

Results: We were able to accurately control HIFU transducer motion using the robotic setup. The HIFU transducer could be moved and rotated in all three orthogonal directions, with respect to either the skin or the HIFU focus.

Conclusions: The feasibility of robotic HIFU transducer control during noninvasive HIFU surgery for atherosclerotic plaque targeting was demonstrated, along with real-time visualization of the ablation positions.

P044-F | Exploiting gene expression data for understanding gender-specificity in hypertrophic cardiomyopathy

A.I.F. Avila; R. Yotti; M.Á. Espinosa; I. Méndez; M. Tamargo; S. Cuenca; F.F. Avilés

Cibercv, HGUGM, Madrid, Spain

Background: Gender is one of the important modifying factors in hypertrophic cardiomyopathy (HCM). For instance, woman with HCM had been reported to be older and more symptomatic than man at the time of their initial diagnosis. However, the information regarding the mechanisms underlying such gender differences is limited. The objective of the current study was to identify genes with significant differential expression between men and women HCM patients, as well as their involvement in physiological pathways.

Material and methods: Public microarray gene expression data (GSE36961) from surgical myectomy from 106 HCM patients (55 woman and 51 man, mean age 46 [9–78]), and 39 controls (14 woman and 25 man, mean age 37 [4–65]) were compared using Class Comparison analysis for gender effect in Babelomics 5.0, and corrected for multiple tests following Benjamini & Hochberg adjustment (FDR < 0·05). Gene Ontology (GO) enrichment was applied to functionally annotate sets of differentially expressed genes.

Results: The gender analysis in HCM patients revealed 66 differentially expressed known genes, however, none significant differences could be detected in healthy samples, supporting the important gender-specific differences in HCM. As expected, many of the differentially expressed genes are X or Y-linked, 16 Y-linked transcripts upregulated in man and 20 X-linked transcripts, 7 upregulated in man and 13 in woman. But most interestingly, 30 transcripts correspond to genes located on autosomal chromosomes, 12 upregulated in woman and 18 upregulated in

man. These genes were found to be significantly overrepresented in the regulation of heart contraction GO term (GO: 0008016; P-value = 9×10^{-4}). Moreover, key genes already known to be responsible of cardiac channelopathies appeared differentially expressed, such as the Calcium channel, voltage-dependent, beta 2 subunit (CACNB2) gene, associated to Brugada syndrome.

Conclusions: The results support the important gender-specific differences in HCM disease and specifically in the regulation of heart contraction.

P045-F | Mitochondrial respiratory changes in pulmonary artery in the monocrotaline pulmonary arterial hypertension model

M. Batlle*,†; A. Power‡; C. Hedges§; T. Jones‡; L. Mont*,†,¶; A. Hickey§; M. Ward‡

*IDIBAPS, Barcelona, Spain; †CIBERCV, Spain; ‡Department of Physiology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand; §School of Biological Science, The University of Auckland, Auckland, New Zealand; ¶Hospital Clinic de Barcelona, Barcelona, Spain

Background: The monocrotaline (MCT) animal model of pulmonary arterial hypertension (PAH) is characterized by hypertrophy of the pulmonary artery (PA). A mitochondrial and metabolic mechanism has been proposed for initiation and maintenance of PAH. The aim of this study was to investigate the respiration chain function and reactive oxygen species (ROS) production in PA and aorta (Ao) from MCT-rats.

Methods: Male Wistar rats were given an I.P. injection of 60 mg/kg of MCT or saline (control rats) and sacrificed after 4 weeks. PA and ascendant Ao were dissected and kept in iced-cold Krebs-Henseleit solution. Both arteries were solubilised with saponin and respiration was measured with oxygraphs at 37°C. A substrate uncoupler inhibitor titration protocol was applied to analyze the respiration flux of different respiratory chain complexes. Data was analyzed with two-way ANOVA or Mann–Whitney U tests with the SPSS17 software.

Results: The MCT-treated animals displayed evidence of right ventricular hypertrophy (right ventricular thickness: Control: $1{\cdot}8 \pm 0{\cdot}3$ mm, MCT: $2{\cdot}3 \pm 0{\cdot}2$ mm, $n = 7$, $P < 0{\cdot}01$). Respiration rates from PA were higher in MCT rats than in controls ($P < 0{\cdot}05$), but no difference was detected in the Ao respiration rates. Pair wise comparisons showed a higher respiration rates in PA of MCT rats than in controls in the oxidative phosphorylation state with complex II (CII-OXPHOS, $P < 0{\cdot}01$), and higher complex I and II leak state flux (CI, CII-Leak, $P < 0{\cdot}01$). Also, lower ETS/OXPHOS and ETS/CI, CII Leak were quantified in the MCT rats. No changes in total ROS were quantified either in PA or in Ao of MCT rats but there was higher ROS normalized by O2 flux in PA from control rats ($P < 0{\cdot}01$).

Conclusions: Increases in mitochondrial respiration were observed in the MCT PA, but not the Ao. These changes are likely to be necessary for the initiation and perpetuation of PAH.

P046-F | Potential role of MMP9 in vascular remodeling triggered by high-intensity exercise. Results of aortic microRNA levels from an exercise murine model

G. Sangüesa*,†; C. Rubies*; M. Batlle*,†; N. Castillo*; G. Fernandez‡; M. Sanz-de-la-Garza§; M. Sitges†,§; J. Brugada†,§; L. Mont†,§; E. Guasch*,†,§

*IDIBAPS, Barcelona, Spain; †CIBERCV, Spain; ‡Bioinformatics Unit, Genetics and Molecular Medicine Service, Hospital Sant Joan de Déu, Esplugues del Llobregat, Spain; §Institut Clínic Cardiovascular, Hospital Clínic, Barcelona, Spain

Introduction: Moderate exercise reduces the atherosclerosis burden, but recent clinical and experimental data suggest that extreme-trained endurance athletes might be at an increased risk of cardiovascular events, likely because of arterial tunica media abnormalities. However, the pathology and mechanisms behind such adverse events remain unclear. In this study we aimed to evaluate miRNA expression profiling as a mediator in intense exercise-induced vascular maladaptation.

Material and methods: Male Wistar rats underwent intense (INT, 60 minutes 60 cm/s, $n = 20$) or moderate treadmill training (MOD, 40 minutes 35 cm/s, $n = 20$) for 16 weeks. Sedentary rats (SED, $n = 20$) served as controls. Fibrosis was quantified in paraffin-embedded aortic sections. Ascending aorta stiffness was estimated in vivo by mean of the beta index through hemodynamic and echocardiographic data. miRNA expression in thoracic aorta was profiled with an Affymetrix rat miRNA 4.1 array, and differentially expressed miRNAs were validated with quantitative PCR (qPCR). Validated targets in TarBase v7.0 were used as the input for Ingenuity analysis.

Results: INT rats exhibited increased tunica media fibrosis ($P < 0{\cdot}01$) and aorta stiffening ($P < 0{\cdot}05$) compared with SED and MOD. miRNA array analyses revealed 21 deregulated miRNAs overall. Amongst them, 4 miRNA shared deregulation in INT vs both MOD and SED groups (miR-132-3p, miR-212-3p, miR-146b-5p, miR-326-5p), of which the first 3 were confirmed with qPCR. Ingenuity analyses

pointed to MMP9 as a central regulator of INT exercise-induced increased vascular stiffness.

Conclusions: miR-132/212 and miR-146b up-regulation might trigger intense exercise-induced vascular fibrosis and stiffening. MMP9 evolves a central enzyme in such process. Our data provides the first available insights into the mechanisms of the potential deleterious vascular consequences associated with very high exercise load.

P047-F | Stimulation of endogenous hydrogen sulfide synthesis contributes to the restoration of cardiac function after ischemia-reperfusion in old animals

L. Mys; N. Strutynska; J. Goshovska; V. Sagach

Bogomoletz Institute of Physiology of Nas of Ukraine, Kiev, Ukraine

Background: Hydrogen sulfide (H_2S) as well as nitric oxide belong to gas transmitters' family and play an important regulatory role in the human body. H_2S is synthesized from aminoacid L-cysteine by cystationin-β-synthase (CBS), cystationin-γ-lyase (CSE) localized in cytoplasm and 3-mercaptopyruvate sulfur transferase, mitochondrial enzyme which is coupled with cysteine aminotransferase enzyme (CAT). CBS, CSE and CAT have pyridoxal-5-phosphate (PLP) as co-factor. The aim of the current work was to study the effect of PLP administration at endogenous hydrogen sulfide synthesis and on the heart function of old rats in Langendorff ischemia-reperfusion model.

Materials and methods: Wistar male rats were divided into 3 groups: adult (6 months), old (24 months) and old + PLP. PLP was administered per os in dose of 0·7 mg/kg daily for 2 weeks. Isolated hearts were perfused by Langendorff technique and underwent ischemia-reperfusion (20 minutes/40 minutes). We evaluated cardiodynamics as left ventricular developed pressure, dP/dt min, dP/dt max, heart rate, and coronary flow. Additionally, content of H_2S was measured in heart tissues.

Results: It has been shown that H_2S content was 2 times lower in old rat heart comparing to adult ones. However, PLP induced significant increase H_2S content in heart tissue. It was found that administration of PLP was accompanied by a significant prevention of reperfusion disorders of cardiac function compared to control rats and contributed to the restoration of myocardial contractile activity of isolated hearts after ischemia.

Conclusions: Our research demonstrated that the 2 weeks administration of PLP might be used for restoration of endogenous H_2S content and prevention of ischemia-reperfusion damage of the heart in old animals.

P048-F | New insights into the role of autophagy in experimental cardiac pathophysiology

A. Planavila; C. Rupérez; G. Ferrer-Curriu; M. Cairó; J. Villarroya; F. Villarroya

Departament de Bioquímica i Biologia Molecular. Institut de Biomedicina de la Universitat de Barcelona (IBUB), University of Barcelona, Barcelona, Spain

Introduction: Autophagy is an intracellular process that mediates protein degradation, organelle turnover, and recycling of cytoplasmic components. Despite the beneficial role of autophagy, excessive or insufficient autophagic activity can each contribute to cell death. Several studies have shown that autophagic flux contributes to the pathogenesis of cardiac diseases. The aim of the study was to gain insight into the role of autophagy in cardiac tissue during different pathophysiological conditions involving energy metabolism challenges to heart.

Methods: Two-month old mice were exposed to different experimental conditions: 12 hours starvation, high-fat diet-induced cardiac hypertrophy (16 weeks), cold-induced cardiac hypertrophy (4°C), and reversion by placing mice into thermo-neutral conditions (30°C) for 24 hours or 1 week. Autophagic events were determined by Western blot of autophagy markers, electron microscopy and autophagic flux determination using leupeptin treatment.

Results: First, we studied the role of autophagy during different dietary conditions. We found that 12 hours starvation induced cardiac autophagic flux in the hearts of mice. By contrast, high-fat diet-induced cardiac hypertrophy blocks autophagy in cardiac tissue and leads to lipid droplet accumulation. Moreover, in this context the protective cardiomyokine fibroblast growth factor-21 (FGF21) leads to activation of cardiac autophagy. Next, we studied the role of autophagy during cardiac hypertrophy in the model of cold-induced hypertrophy and its reversion after deacclimation. We found that cardiac autophagy was repressed in cold-induced cardiac hypertrophy, and re-activated during the first 24 hours after returning to thermo-neutral conditions. The autophagic flux, determined by assessment of LC3b II after leupeptin treatment in vivo, was repressed during cold exposure and strongly reactivated after 24 hours of thermoneutrality.

Conclusions: We propose autophagy as a key component of nutrient-sensing machinery in the heart and a relevant mechanism controlling cardiac remodeling. Modulation of autophagy by FGF21 might be a therapeutical target to treat cardiac diseases.

P049-F | H_2S donor (NaHS) attenuates age-associated diastolic dysfunction by oxidative/nitrosative stress inhibition and cNOS recoupling.

K. Drachuk*,†; N. Dorofeyeva*; V. Sagach*

*Bogomoletz Institute of Physiology, Nas Ukraine, Kyiv, Ukraine; †National O.O. Bohomolets Medical University, Kyiv, Ukraine

Background: The effects of H_2S donor (NaHS) on oxidative/nitrosative stress markers, coupling of constitutive NO-synthase (cNOS) and hemodynamic parameters in old rats were investigated.

Material and methods: Hemodynamic parameters were studied using the Millar pressure–volume (P–V) conductance catheter system in vivo. The markers of oxidative/nitrosative stress (the rate of O_2•−, •OH generation, the activity of iNOS) and constitutive NO-synthesis (activity of cNOS and NO_2− pools) were determined in heart tissue by spectrophotometric method. The index of cNOS coupling was calculated as cNOS activity related to the rate of O_2•−generation.

Results: It was found the impaired diastolic function in old rats (decrease of the rate of the left ventricle relaxation [dp/dtmin] by 33%, 3-times increase of the end-diastolic pressure [EDP], an increase of the time constant of left ventricular relaxation [Tau g] by 44% and 2-time increase of the end-diastolic stiffness [EDS]). Diastolic dysfunction was accompanied by a decrease of H_2S pools (by 1·9 times). Simultaneously, oxidative/nitrosative stress was developed, leading to cNOS uncoupling (the index of cNOS coupling was decreased by 7·5 times) and decline of the constitutive NO synthesis (cNOS activity and NO_2− pools were decreased by 2·1 and 1·6 times, respectively).
The NaHS injection improved diastolic function in old rats (dP/dtmin increased by 20% and the Tau g decreased by 13%). The molecular mechanisms of NaHS action included the increase of H_2S pools (by 3·3 times). The latter was accompanied by inhibition of the oxidative/nitrosative stress, cNOS recoupling (index of cNOS coupling increased by 19 times) and reduction of the constitutive NO synthesis (cNOS activity and NO_2− pools were increased by 2·5 and 3·8 times, respectively).

Conclusions: Thus, NaHS improves diastolic function in old rats via oxidative/nitrosative stress inhibition, cNOS recoupling and constitutive synthesis of NO stimulation.

P050-F | Age-dependence of aortic geometry in Marfan syndrome: a 4D-flow CMR study

A. Guala; G. Teixido-Tura; J. Rodriguez-Palomares; L. Dux-Santoy; A. Ruiz-Munoz; I. Dentamaro; N. Villalva; F. Valente; L. Galian; L. Gutierrez; C. Granato; T. Gonzalez-Alujas; R. Fernandez; A. Sao-Aviles; D. Garcia-Dorado; A. Evangelista

Department of Cardiology, Hospital Vall D'hebron, VHIR, Universitat Autònoma De Barcelona, Barcelona, Spain

Introduction: Despite the main anatomical change in Marfan syndrome (MFS) is dilatation of the aortic root, the whole thoracic aorta is affected. A wide range of age dependences have been proven by studies of 3D aortic geometry in healthy subjects and in different pathologies. However, a 3D analysis of the effect of ageing on thoracic aorta in MFS is missing.

Purpose: We aimed to evaluate 3D aortic geometry evolution with age in MFS by 4D-flow CMR.

Methods: 30 patients with MFS were studied. Anatomical 3D images (MRA) were obtained through non-contrast enhanced 4D-flow and were used to characterize thoracic aortic geometry by means of a semi-automatic segmentation. Five sections (sinotubular junction (STJ), ascending (AAo) and descending (DAo) aorta at pulmonary level and first and last supra-aortic vessels) divided the aorta into 4 segments: proximal and distal AAo, aortic arch, and proximal DAo. For each volume mean diameter, length, tortuosity, curvature and volume were extracted. Sagittal depth was measured as the maximum depth of aortic arch perpendicular to the line connecting AAo and DAo at the level of the pulmonary artery.

Results: Diameter, lengths and volumes of AAo, arch and DAo increase with age (all $P < 0{\cdot}05$). Proximal AAo becomes more curved and tortuous with age while the opposite happens to distal AAo (all $P < 0{\cdot}05$). AAo maximum curvature location moves proximally with ageing ($P = 0{\cdot}001$). Aortic arch becomes straighter, higher and wider and sagittal depth increases with age (all $P < 0{\cdot}05$). STJ angulation is not impacted by age.

Conclusions: In MFS, the whole aorta undergoes complex 3D-geometrical changes that comprise much more than the dilatation of the aortic root. AAo, aortic arch and DAo shape, tortuosity and curvature highlighted a complex 3D pattern. Further studies are needed to characterize their eventual clinical implications.

P051-F | Effect of spider silk matrix on cardiac tissue regeneration of mesenchymal stem cells

L. Gomez-Cid*; L. Fuentes*; M.E. Fernandez-Santos*; S. Suarez-Sancho*; V. Plasencia*; A.M. Climent*; R. Sanz-Ruiz*; M. Hedhammar†; F. Atienza*; F.F. Aviles*

Hospital GU Gregorio Marañon, Servicio Cardiología, CIBERCV, Madrid, Spain; †KTH School of Biotechnology, AlbaNova University Center, Stockholm, Sweden

Background: Cell therapy for heart diseases face the hostile environment for cell retention and survival in the area were the cells are delivered. In order to minimize the impact the poor environment has on the implanted cells, functionalized spider silk matrices are proposed as cell vehicles for the infarcted region. Recombinant spider silk proteins (spidroins) are capable of self-assembling into fibres in aqueous solutions and can create very thin and resistant matrices with interesting mechanical properties.

Material and methods: In this study, functionalization of spider silk matrices with fibronectin and vitronectin was carried out to improve cytocompatibility, as these two extracellular matrix proteins are associated to cell adhesion and retention. Mesenchymal Stem Cells (MSC) were cultured for 8 days on these matrices and we compared the ability of the cells to grow and adhere in monolayers and their proliferation rates. We further studied the impact of cell-matrix interactions in cell behaviour by analysing the rate of cell migration and changes in cell secretion during the last 48 hours of culture.

Results: MSC growing rate and adhesion of functionalized matrices was higher than in non-functionalized matrices, being vitronectin the most effective protein promoting cell growth. Moreover, analysis of the three cropped secretomes showed that vitronectin functionalization also enhanced exocytosis considerably.

Conclusions: These results show that vitronectin functionalized spider silk matrices are a preferred option to be tested as scaffold matrices for preclinical studies on small animal models, as they enhance the regenerative profile of stem cells.

P052-F | Drug timing optimization in arterial hypertension treatment: one step towards personalized blood pressure control based on the individual circadian blood pressure profile

O. Mamontov*,†; O. Markelov‡; M. Bogachev‡,§

Almazov National Medical Research Centre, Saint Petersburg, Russian Federation; †Pavlov First State Medical University, Saint Petersburg, Russian Federation; ‡St. Petersburg Electrotechnical University, Saint Petersburg, Russian Federation; §Kazan Federal University, Kazan, Russian Federation

Background: Considerable elevation of the absolute blood pressure (BP) values in patients with complicated arterial hypertension is often accompanied by disruptions of their circadian BP profile.

Materials and methods: The circadian profile was assessed from the BP data recordings over three consecutive days that were subsequently folded according to its clock hours and fitted by a gliding 3rd order polynomial. In every patient the AHT was optimized individually to inhibit the most pronounced BP increases above the target level during the diurnal cycle. The drug timing has been adjusted such that the maximal concentrations of the antihypertensive agents coincided with the clock time when the first derivative of the profile curve started increasing. BP monitoring sessions were repeated in 2 weeks to evaluate the effect of the adjusted treatment scheme.

Results: Significant positive dynamics in the circadian BP profile could be observed as a result of the drug timing correction. In particular, the number of patients with normal nocturnal BP reduction (dippers) increased from 24 to 39 after therapy adjustment, while the number of patients with insufficient reduction or even increase of nighttime BP (non-dippers and night-peakers), reduced from 27 to 12 patients ($P < 0{\cdot}05$). Remarkably, after AHT optimization the total number of the antihypertensive agents increased from 2·4 to 2·8 ($P < 0{\cdot}05$), while the number of daily medication intakes did not change significantly.

Conclusion: We have suggested and validated a systematic approach to the personalized antihypertensive therapy (AHT) optimization based on the features of the individual circadian BP profile of each patient. Our data indicate that optimization of drug timing resulted in significant improvement of the AHT efficacy.

P053-F | Blood pressure – heart rate synchronization: a complementary indicator of the baroreflex mechanism activity?

N. Pyko*; S. Pyko*,†; O. Markelov*; O. Mamontov†,‡; M. Bogachev*,§

St. Petersburg Electrotechnical University, Saint Petersburg, Russian Federation; †Almazov National Medical Research Centre, Saint Petersburg, Russian Federation; ‡Pavlov First State Medical University, Saint Petersburg, Russian Federation; §Kazan Federal University, Kazan, Russian Federation

Background: Baroreflex is one of the key mechanisms of short-term blood pressure feedback regulation that governs responses to various physical and mental stresses. While BRS quantifies explicitly the heart rate response to the variations of the blood pressure, it does not contain any

information whether such a response was activated for every significant of blood pressure variation.

Materials and methods: We analysed blood pressure – heart rate synchronization dynamics under both stationary and orthostatic stress test conditions. To detect the episodes of synchronous behaviour, we employed a technique based on the instantaneous phase analysis assessed by Hilbert transform. We consider the synchronization coefficient Sync defined as the fraction of the time intervals where the standard deviation of the instantaneous phase difference is below a given threshold. We suggest that Sync could be used as an additional indicator of the heart rate – blood pressure mechanism efficacy that is complementary to the widely used BRS. While the BRS value characterizes the intensity of the heart rate response to the changes in blood pressure, the Sync value indicates how often such response is activated in the first place.

Results: Based on the analysis of 67 orthostatic tilt test records, we have shown explicitly that in both healthy subjects and patients with moderate autonomic dysfunction BRS and Sync are reciprocal indicating that lower BRS tend to be compensated by a more frequent activation of the baroreflex loop characterized by higher Sync (Corr = −0·43, $P < 0·001$). In contrast, in diabetes patients with autonomic neuropathy BRS and Sync are positively correlated likely indicating the breakdown of this compensation mechanism.

Conclusion: We therefore suggest that the BRS*Sync product could appear a useful and complementary indicator of the overall baroreflex mechanism efficacy.

P054-F | Action potential duration and conduction velocity as predictors of ventricular tachycardia inducibility

I.H. Romero*; A. Liberos*; Á. Arenal*; P.M. Ruiz-Hernández*; V. Crisóstomo†; R. Sánz*; F. Atienza; M.S. Guillém‡; J. Bermejo*; F.M. Sánchez-Margallo†; A.M. Climent*; F. Fernández-Avilés*

**Hospital General Universitario Gregorio Marañón and CIBERCV, Madrid, Spain; †Centro de Cirugía de Mínima Invasión Jesús Usón, Cáceres, Spain; ‡ITACA, Universitat Politécnica de Valencia, Valencia, Spain*

Introduction: The incidence of ventricular tachyarrhythmias increases within the few months after acute myocardial infarction. Identification of proarrhythmic substrate characteristics will help in the stratification of chronic infarct patients depending on the risk of suffering a ventricular arrhythmia. In this work action potential duration (APD) and conduction velocity (CV) are evaluated as predictors of inducibility in a chronic infarction swine model.

Methods: In 21 swine, myocardial infarction was induced by occlusion-reperfusion sequence in the left anterior descending coronary artery. After 16 weeks of myocardial infarction, animals were tested under a programmed electrical stimulation, and sacrificed to explant the heart in a perfused Langendorff system where optical mapping of the electrical activity was performed. Action potential duration (APD80) and conduction velocity (CV) were measured during epicardial right ventricle pacing. Measures were grouped by zones to distinguish between the distal regions and the heterogeneous tissue present at the scar border zone, and compared between the successes of induction.

Results: Electrophysiological differences were observed in APD80s of distal region of 179·89 ± 39·20 ms in the case of positive induction vs 234·75 ± 27·86 ms without a success induction ($P < 0·05$), and in the heterogeneous tissue of 213·95 ± 49·63 ms vs 260·00 ± 28·80 ms ($P < 0·05$) for the same groups. CV differences were observed in distal region 68·57 ± 9·86 cm/s in the case of positive induction vs 85·55 ± 8·90 cm/s without a success induction ($P < 0·05$), meanwhile CV of heterogeneous tissue did not show differences.

Conclusions: Initiation and maintenance or ventricular tachycardia is associated with shorter APDs in both distal and scar border zone regions, and slower CVs in distal regions from infarction. The quantification of these parameters by means of invasive or non-invasive techniques will improve stratification of chronic infarction patients and thus the prevention strategies of such arrhythmias.

P055-F | Thrombus aspiration in ST-segment-elevation myocardial infarction. Safety and 1-year outcomes. A single center experience: CardioCHUS MI registry

A.R. Diéguez; B.C. Alvarez; R.T. Nouche; A.A. Carrillo; F.G. Peña; B.A. Alvarez; X.S. Pena; D.L. Otero; J.R.G. Juanatey

Chus (Complexo Hospitalario Univeristario de Santiago de Compostela), CiberCV, Santiago De Compostela, Spain

The latest evidence on thrombus aspiration (TA) in STEMI patients undergoing PPCI suggests that TA does not improve outcomes and is associated with a high risk of stroke. Nonetheless, it is still a common practice for patients with a significative thrombus burden.

Objectives: Our aim was to assess the effect of TA in the risk of acute TIA/stroke, and in one-year prognosis in terms of all-cause death and major adverse cardiac events. (MACE) (all-cause mortality, myocardial infarction, and unplanned repeat revascularization)

Methods and results: From January 2008 to December 2016, 1723 patients (86·4% male, the mean age of 58·4 years) were admitted to our hospital with STEMI and underwent PPCI. TA was performed in 1178 patients (68·1%).

Baseline characteristics were significative different between TA and no TA patients: TA patients were younger, more frequently males and on smoking, had fewer comorbidities (hypertension, diabetes, dyslipidemia, renal insufficiency and ischemic heart disease), the infarct-related artery was less frequently the LAD and had a lower pre-PCI TIMI flow. After propensity score matching no differences among groups were detected.

124 patients died (7·2%) and 269 patients presented MACE (15·6%) during follow up. In the total cohort, there were no differences in the incidence of TIA/stroke (1·55% in TA patients vs 1·44%, *P* 0·871) this was maintained in the PS-matched cohort (1·56% in TA patients vs 1·07%, *P* 0·300).

No differences were observed in 7 days, 30 days or one-year all-cause mortality and MACE among TA and No-TA groups in both cohorts (total and PS-matched cohort)

Conclusion: In our contemporary STEMI registry the use of TA in PPCI was safe in terms of acute TIA/Stroke, however no outcome benefit was detected at one year.

P056-F | Carcinoid heart disease; the importance of a multidisciplinary heart team

E.A. Hart; R.C. Meijer; M. Tesselaar; A. Tuinenburg; R.S. van Leeuwaarde; S.A. Chamuleau

Umc Utrecht, Utrecht, The Netherlands

Background: Neuroendocrine tumors are able to secrete vasoactive substances which may result in carcinoid syndrome. The cardiac manifestations of neuroendocrine tumors are known as carcinoid heart disease and mostly affects right-sided valves, leading to severe regurgitation and/or stenosis and subsequent cardiac decompensation. Treatment consist of medical therapy and/or surgical intervention in selected cases.

Materials and methods: Here we present an illustrative case report of a relatively young patient with carcinoid heart disease. This case highlights the importance of a multidisciplinary heart team in choosing optimal treatment strategy.

Results: A 45-year old female with carcinoid heart disease underwent tricuspid and pulmonic valve replacement with a bioprosthesis. Postoperatively she developed a third degree atrioventricular block for which she received a pacemaker. The ventricular lead was placed in a posterolateral branch of the coronary sinus. Shortly thereafter she developed severe mitral regurgitation apparently caused by pacing of the papillary muscle. A new ventricular lead was placed anteriorly in the great cardiac vein, resulting in a significant reduction of mitral regurgitation. One year later she was readmitted with clinical signs suspect for recurrent carcinoid heart disease. She underwent successful tricuspid valve replacement with a mechanical valve. Microscopic analysis confirmed recurrent carcinoid heart disease.

Conclusions: In this case report we present a complex disease which requires a multidisciplinary approach. Decisions with regards to valve type (bioprosthesis or mechanical valve) and treatment of complications (atrioventricular block, mitral regurgitation, recurrent heart disease) ought to be made by a dedicated heart team, therefore early referral to a specialized center are of importance. An imaging cardiologist, an electrophysiologist, and a cardiothoracic surgeon were closely involved in the process, which illustrates the crucial role of a heart team in the treatment of patients with this complex disorder.

P057-F | Fracture of the sesamoid bone of the thumb: a case report and summary on literature

N. van der Naald*; M. van der Naald†; A. Schuurman†

**St Antonius Hospital, Nieuwegein, The Netherlands; †University Medical Center Utrecht, Utrecht, The Netherlands*

A fractures of the sesamoid bone of metacarpophalangeal joint is rare. Here we present a case of a 26-year old female patient with a hyperextension trauma of the thumb causing an isolated, complete fracture of the ulnar sesamoid bone of the thumb MCP joint. Injury of the volar plate was excluded. In an additional systematic literature search we found 40 cases. In 73% the ulnar sesamoid bone was fractured, and in 24% an isolated fracture of the radial sesamoid bone occurred. The injury most often occurs during sports (50%). Immobilisation was the therapy of choice in 80% of the cases. In most of these cases immobilization was <4 weeks (78%) or even <3 weeks (63%). Full recovery was established in 91% of the cases, of which 50% recovered <8 weeks. Physicians should be aware of this uncommon trauma. We advise a short period of immobilisation, not more than 3 weeks, and early physical treatment if required.

P059-F | Calix[4]arene methylene bisphosphonic acids as promising inhibitors of plasmin fibrinolytic activity

T. Yatsenko; T. Grinenko; O. Savchuk; O. Yusova

Palladin Institute of Biochemistry of NAS of Ukraine, Kyiv, Ukraine

Background: Since wide range of hemorrhagic disorders is related to plasminogen over activation search of effective specific low molecular weight plasmin inhibitors is relevant research issue. Calixarenes are perspective class of low-toxic compounds which can be functionalized and used as biologically active substances due to their ability to form supramolecular complexes with biological molecules.

Materials and methods: We have assessed the ability of series of calix[4]arenes functionalized by various number of phosphonic acid remnants to inhibit fibrinolysis (human plasmin amidolytic and fibrinolytic activity, plasminogen activators activity).

Results: Calix[4]arenes C 296, C-425, C-427 and C-145 with respectively 2, 4, 3 and 8 phosphonic groups, specifically inhibit fibrin clot lysis by plasmin in dose-dependent manner. Number of phosphonic acid remnants in calixarene structure is proportional to suppression effect on plasmin activity. C-145 (sodium salt of calix[4]arene-methylene-bis-phosphonic acid) have shown most effective plasmin inhibition by competitive mechanism (Ki = 0·26 μM). At the same time ¼ part of the calixarene – 4-hydroxyphenyl-methylene-bis-phosphonic acid – do not effect plasmin fibrinolytic activity, that indicates the requirement of whole calix(4)arene molecule for the enzyme inhibition. However, C-145 does not effect amidolytic plasmin activity toward chromogenic substrate that is low molecular weight substance. Probably such calix(4)arene selectivity to fibrillary substrate like fibrin is provided by complex formation between 8 negatively charged phosphonic groups in upper ring and positively charged substrate recognition exosites of plasmin active site.

Conclusions: Our results demonstrate that C-145 is perspective as potential novel pharmaceutical agent for clinical intervention against fibrinolytic system overactivation disorders.

P060-F | Pro B-type natriuretic peptide strongly predicts all cause and cardiovascular mortality in peripheral arterial disease patients with as well as in those without type 2 diabetes

A. Mader*,†,‡

**Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; ‡Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria*

Background: Pro B-type natriuretic peptide (proBNP) is an established prognostic biomarker in patients with heart failure. Its power to predict all-cause and cardiovascular mortality in peripheral arterial disease (PAD) patients with type 2 diabetes (T2DM) is unclear and is addressed in the present study.

Material and methods: We prospectively investigated a consecutive series of 309 patients with sonographically proven PAD. Presence of T2DM was diagnosed according to the current ADA criteria. Mortality was recorded over a follow-up period of 6 years.

Results: At baseline, proBNP was significantly higher in PAD patients with T2DM (n = 134) than in those who did not have T2DM (1230 ± 3541 pg/mL vs 879 ± 3730 pg/mL; P = 0·017). During follow-up, both among PAD patients with T2DM and among PAD patients without T2DM all-cause mortality (46·3 % and 26·2%; P = 0·001) and cardiovascular mortality (17·9% and 11·4%; P = 0·166) were high. Serum proBNP after multivariate adjustment significantly both in patients with T2DM and in those without T2DM predicted all-cause (HRs 1·364 [1·29–2·07]; P < 0·001 and 2·51 [1·62–3·89]; P < 0·001, respectively) as well as cardiovascular mortality (HRs 1·38 [1·02–1·86]; P = 0·036, and 1·61 [1·24–2·10]; P < 0·001, respectively).

Conclusion: We conclude that proBNP strongly predicts all-cause and cardiovascular mortality in PAD patients with T2DM as well as in nondiabetic PAD patients.

P061-F | Pro-B-type natriuretic peptide strongly predicts cardiovascular mortality in coronary artery disease patients with type 2 diabetes

D. Zanolin*; C.H. Saely*,†,‡,§; A. Vonbank*,†,‡; C. Lins¶; A. Leiherer*,†; A. Mader*,†,‡; B. Larcher*,†,‡; A. Muendlein*,†; H. Drexel*,†,§,**

*Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; ‡Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; §Division of Angiology, Swiss Cardiovascular Center, Berne, Switzerland; ¶Kantonsspital St. Gallen, Switzerland; **Drexel University College of Medicine, Philadelphia, USA

Background: Elevated pro-B-type natriuretic peptide (proBNP) is associated with an increased risk of cardiovascular events in various populations including patients with type 2 diabetes (T2DM) and patients with coronary artery disease (CAD). The power of this biomarker to predict cardiovascular mortality in patients with the combination of T2DM and CAD is unclear and is addressed in the present study.

Material and methods: We prospectively investigated a consecutive series of 591 patients with angiographically proven CAD over a mean follow-up period of 5·9 ± 1·1 years.

Results: At baseline, proBNP was significantly higher in patients with T2DM (n = 163; 27·6% of the study population) than in nondiabetic subjects (793 ± 1249 vs 685 ± 1401 pg/mL; P = 0·020). Prospectively, cardiovascular death occurred significantly more frequently in patients with T2DM than in nondiabetic subjects (14·1% vs 6·3%; P = 0·002) and cardiovascular death strongly increased over tertiles of proBNP in patients with T2DM (4·3%, 21·7%, and 73·9%, respectively; P = 0·019) as well as in subjects without T2DM (11·1%, 14·8%, and 74·1%, respectively; P < 0·001). Concordantly, serum proBNP significantly predicted cardiovascular mortality after adjustment for age, gender, smoking, LDL cholesterol, HDL cholesterol, hypertension, and eGFR both in patients with T2DM (standardized adjusted HR 2·36 [1·48–3·77]; P < 0·001) and in those without T2DM (HR 1·59 [1·19–2·11]; P = 0·002).

Conclusion: We conclude that serum proBNP strongly predicts cardiovascular mortality in CAD patients with T2DM as well as in nondiabetic CAD patients.

P062-F | Serum proBNP predicts a decline in kidney function independently of type 2 diabetes, the baseline kidney function and baseline coronary artery disease

C.H. Saely*,†,‡,§; C. Lins¶; D. Zanolin*; A. Leiherer*,†; A. Vonbank*,†,‡; A. Mader*,†,‡; B. Larcher*,†,‡; A. Muendlein*,†; H. Drexel*,†,§,**

*Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; ‡Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; §Division of Angiology, Swiss Cardiovascular Center, Berne, Switzerland; ¶Kantonsspital St. Gallen, Switzerland; **Drexel University College of Medicine, Philadelphia, USA

Background: Elevated pro-B-type natriuretic peptide (proBNP) is a marker of cardiovascular event risk in various patient populations including patients with type 2 diabetes (T2DM) and patients with coronary artery disease (CAD). Whether proBNP also predicts a decline in kidney function is not known and is addressed in the present study.

Material and methods: Both at baseline and after 4 years of follow-up we assessed kidney function in 462 patients with angiographically proven coronary artery disease (CAD).

Results: At baseline, estimated glomerular filtration rate (eGFR) significantly decreased over tertiles of proBNP (82 ± 20, 80 ± 19, 74 ± 22 mL/min/1·73 m^2; P = 0·003). Further, serum proBNP significantly predicted a decline in eGFR from baseline to after 4 years after adjustment for age, gender, and baseline eGFR (F = 7·80; P = 0·005). The power of proBNP to predict a decline in kidney function was not attenuated after further adjustment for angiographically determined CAD (F = 7·90; P = 0·005) nor after additional adjustment for the presence of T2DM (F = 7·57; P = 0·006).

Conclusion: We conclude that serum proBNP predicts a decline in kidney function independently from T2DM, the baseline kidney function, and baseline CAD.

P063-F | Serum uromodulin predicts a decline in kidney function independently from the presence of type 2 diabetes

A. Leiherer*,†,‡; A. Muendlein*,†; C.H. Saely*,†,§,¶; E.M. Brandtner*; K. Geiger*; A. Mader*,†,§; B. Larcher*,†,§; P. Fraunberger†,‡; H. Drexel*,†,¶,**

*Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; ‡Medical Central Laboratories, Feldkirch, Austria; §Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; ¶Division of Angiology, Swiss Cardiovascular Center, Berne, Switzerland; **Drexel University College of Medicine, Philadelphia, USA

Background: Uromodulin is the most abundant protein excreted in urine. Low uromodulin has been found to be associated with type 2 diabetes (T2DM) as well as with chronic kidney disease (CKD). Whether it also predicts a future decline in kidney function is not known and is addressed in the present study.

Material and methods: We measured serum uromodulin in 529 patients undergoing coronary angiography for the evaluation of established or suspected coronary artery disease (CAD).

Results: Uromodulin was lower in patients with T2DM than in non-diabetic subjects (148 ± 70 vs 171 ± 79; $P = 0{\cdot}001$) and significantly correlated with estimated glomerular filtration rate (eGFR; $r = 0{\cdot}242$, $P < 0{\cdot}001$) and, inversely, with the albumin creatinine ratio (ACR; $r = -0{\cdot}120$, $P = 0{\cdot}012$). It was significantly lower in patients with CKD (eGFR <60 mL/min/1·73 m^2) than in those with normal kidney function (72 ± 29 vs 169 ± 76 ng/mL; $P < 0{\cdot}001$), and also in patients with albuminuria than in patients without increased albumin excretion (149 ± 72 vs 168 ± 78 ng/mL; $P = 0{\cdot}008$). Further, uromodulin at baseline was significantly lower in patients who developed an eGFR <60 mL/min/1·73 m^2 during 4 years of follow-up compared to those who did not (127 ± 42 vs 180 ± 79 ng/mL, $P = 0{\cdot}003$). It was inversely associated with declining eGFR even after full adjustment including ACR, baseline CAD and the presence of T2DM (OR = 0·354 [95% CI 0·131–0·957], $P = 0{\cdot}041$). The inclusion of uromodulin to a basic prediction model for CKD increased the model performance (*C*-statistic 0·844 vs 0·804, $P = 0{\cdot}049$).

Conclusion: In conclusion, we for the first time show that serum uromodulin predicts a decline in kidney function independently from conventional risk factors including T2DM.

P064-F | The creatinine to uromodulin ratio in serum predicts major cardiovascular events independently from the presence of type 2 diabetes

A. Leiherer*,†,‡; A. Muendlein*,†; C.H. Saely*,†,§,¶; J. Ebner*,†; E.M. Brandtner*; A. Mader*,†,§; B. Larcher*,†,§; P. Fraunberger†,§; H. Drexel*,†,¶,**

*Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; ‡Medical Central Laboratories, Feldkirch, Austria; §Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; ¶Division of Angiology, Swiss Cardiovascular Center, Berne, Switzerland; **Drexel University College of Medicine, Philadelphia, USA

Background: Low concentrations of the kidney protein uromodulin are associated with type 2 diabetes (T2DM) and with chronic kidney disease (CKD). The serum creatinine to uromodulin ratio recently has attracted interest as a marker of CKD. Whether this ratio also is associated with the risk for major cardiovascular events is unknown and is addressed in the present study.

Material and methods: We measured uromodulin in 529 coronary patients and prospectively recorded major cardiovascular events (coronary death, fatal and non-fatal ischemic stroke, and non-fatal myocardial infarction) over up to 8 years.

Results: During follow-up, a total of 91 major cardiovascular events occurred. The incidence of major cardiovascular events was significantly higher in patients with T2DM ($n = 141$) than in those who did not have diabetes (25·4% vs 14·6%; $P = 0{\cdot}004$). The creatinine to uromodulin ratio significantly predicted major cardiovascular events both univariately (HR 1·37 [95% CI 1·21–1·56], $P < 0{\cdot}001$) and after multivariate adjustment including the presence of T2DM (HR 1·36 [CI 1·18–1·58], $P < 0{\cdot}001$).

Conclusion: In conclusion, this study for the first time shows that the serum creatinine to uromodulin ratio predicts major cardiovascular events independently from conventional risk factors including the presence of T2DM. Given that the biological role of uromodulin is still elusive this result appears important and may stimulate future research on uromodulin.

P065-F | Type 2 diabetes, chronic kidney disease, and mortality in patients with established cardiovascular disease

C.H. Saely*,†,‡,§; A. Vonbank*,†,‡; C. Lins¶; D. Zanolin*; A. Leiherer*,‡; A. Mader*,†,‡; B. Larcher*,†,‡; A. Muendlein*,‡; H. Drexel*,‡,§,¶

*Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; ‡Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; §Division of Angiology, Swiss Cardiovascular Center, Berne, Switzerland; ¶Kantonsspital St. Gallen, Switzerland; **Drexel University College of Medicine, Philadelphia, USA

Background: Both type 2 diabetes (T2DM) and chronic kidney disease (CKD) are associated with a high risk of cardiovascular disease (CVD) and premature death. We aimed at investigating the single and joint effects of T2DM and of CKD on all-cause mortality in high-risk patients with established CVD.

Material and methods: We prospectively investigated 2108 patients with established CVD (1789 with angiographically proven coronary artery disease and 319 with sonographically proven peripheral artery disease) over 7·0 ± 2·7 years.

Results: Deaths occurred more frequently in T2DM patients (n = 652) than in non-diabetic subjects (38·2% vs 19·6%; $P < 0·001$) and in patients with CKD (eGFR <60 mL/min/1·73 m²; n = 357) than in those with an eGFR ≥60 mL/min/1·73 m² (48·8% vs 19·8%; $P < 0·001$). When both, T2DM and CKD were considered, 1248 subjects had neither T2DM nor CKD, 503 had T2DM but not CKD, 208 did not have diabetes but had CKD, and 149 had both diabetes and CKD. When compared with mortality among patients with neither T2DM nor CKD (16·1%), mortality was significantly higher in patients with T2DM who did not have CKD (30·5%; $P < 0·001$) as well as in non-diabetic patients with CKD (40·1%; $P < 0·001$) and was highest in patients with both, T2DM and CKD (62·4%; $P < 0·001$), in whom mortality was higher than in those with T2DM but no CKD ($P < 0·001$) or those without T2DM but with CKD ($P = 0·045$); mortality was higher in non-diabetic CKD patients than in diabetic patients who did not have CKD ($P = 0·013$).

Conclusion: We conclude that CKD in patients with established CVD confers an even higher mortality risk than T2DM. Mortality is extremely high in CVD patients with the combination of CKD and diabetes.

P066-F | Organ-specific tissue manifestations based on mutations from panel in pediatric onset dilated cardiomyopathy

A. Sammani*; A. Baas†; L. van der Pol†; H. Breur†; G. Visser†; F. Asselbergs*; M. Harakalova*

*Umc Utrecht, Utrecht, The Netherlands; †Wilhelmina Childrens' Hospital, Utrecht, The Netherlands

Background: Dilated cardiomyopathy (DCM) in children commonly leads to overt heart failure and the need for heart transplantation, while myopathies result in progressive muscle weakness and disability. Both however, can share common genetic etiology. Since (cardio)myopathies can be part of distinct syndromic disorders, present diagnostic tests underrepresent the whole genetic spectrum and other organ manifestations are neglected. Based on pilot data, we hypothesize presence of organ manifestation in DCM correlates with organ specificity of mutated genes.

Material and methods: Data was retrospectively gathered at the Wilhelmina Children's Hospital (WKZ) and the University Medical Center Utrecht, The Netherlands. All patients with pediatric onset of DCM were included and phenotyped on neurological, cardiological, nephrological and ophthalmological basis, and dysmorphology. If performed genetic testing was inconclusive, patients were referred to geneticist for a step-up approach using panel whole-exome sequencing (WES) or whole genome sequencing (WGS). Mutations were correlated to organ specific expression patterns based on public RNAseq datasets.

Results: 49 cases of pediatric onset DCM were included, of which 9 reached a (cardiac) end-point such as heart transplantation or death. Twelve patients had conclusive pathogenic mutations, whereas 19 patients needed diagnostic re-evaluation and are awaiting diagnostic panel or WES. In 3 patients, trio-WES revealed no conclusive genetic diagnosis. Thirteen patients had extra-cardiac manifestations. Based on a panel of 64 cardiomyopathy associated genes, the tissue-specific expression pattern of two cases correlated to specific organ manifestations.

Conclusions: In our cohort, mutated genes found on a cardiomyopathy panel, showed low correlation to additional organ manifestations. However, WES can ameliorate the yield and provide explanation for pediatric heart failure. Using additional WES-data, tissue specificity will be correlated in patients with extra-cardiac manifestations in further research.

P067-F | Mechanism of antiplatelet action induced by mitoq via mitochondrial protection

E. Fuentes*; R. Araya-Maturana†; D. Arauna*; M. Fuentes*; I. Palomo*

*Thrombosis Research Center, Department of Clinical Biochemistry and Immunohaematology, Faculty of Health Sciences, Interdisciplinary Excellence Research Program on Healthy Aging (PIEIES), Talca, Chile; †Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile

Background: Mitoquinone mesylate (MitoQ) is a mitochondria-targeted antioxidant designed to accumulate within mitochondria to protect against oxidative damage. The active antioxidant component of MitoQ is ubiquinone and this approximately thousand-fold greater concentration of MitoQ within mitochondria that makes it more effective at preventing mitochondrial oxidative damage. MitoQ exhibits protective activity against liver fibrosis, hepatic oxidative stress, portal hypertension, thrombocytopenia induced by irradiation, and ototoxicity induced by amikacin and cisplatin; further an anti-inflammatory, anticarcinogenic and cardioprotective effect. It has been described that high levels of oxidative mitochondrial stress (due to aging, cardiovascular diseases, and others) alter the platelet metabolism, both at ATP production and at functional level (hyperactivation), increased the risk of thrombotic events. However, the potential antiplatelet effect of this antioxidant and the specific mechanisms involved have not been studied.

Aims: The main aim of this work was to investigated antiplatelet action mechanisms of antioxidant MitoQ.

Methods: MitoQ (0·01–10 μmol/L) was evaluated in a model of mitochondrial damage by rotenone (20 μmol/L) on (i) Platelet P-selectin expression and by flow cytometry, (ii) Platelet aggregation induced by ADP and convulxin, (iii) platelet activation (change form and platelet size) by fluorescence microscopy, (iv) Intraplatelet reactive oxygen species (ROS) level by probe H2DCFA-DA and flow cytometry, (v) Quantification of active caspase-3 and -7, (vi) release of Cytochrome C by Western Blot, and (vii) cytotoxicity evaluation by LDH activity.
The protocols were authorized by the ethics committee of the Universidad de Talca in accordance with the Declaration of Helsinki.

Results: MitoQ concentration-dependently (0·1–10 μmol/L) inhibited P-selectin expression and aggregation induced by ADP and convulxin in the model mitochondrial damage. Similarly, MitoQ prevents medium platelet size increase and filopodia emission in this model. At these concentrations, MitoQ significantly decreased the ROS levels and release of cytochrome c induced by convulxin (only agonist that induce an increase of ROS intraplatelet level). Although, MitoQ not prevent the release of active caspases. MitoQ presented a cytotoxicity effect in platelet at concentrations 1–10 μmol/L, but at a concentration 0·1 μmol/L maintains its protective effects without cytotoxicity. Moreover, MitoQ had a good antiplatelet effect.

Summary/conclusion: MitoQ presents a remarkable antiplatelet effect by a mitochondrial protective mechanism, but not clear that this effect is dependent of ROS production.

Keywords: MitoQ, antiplatelet, mitochondria, ROS.

Acknowledgements: CONICYT/FONDECYT No. 1180427, CONICYT/REDES No. 170003 and CONICYT/REDES No. 170002. This work was also funded by Interdisciplinary Excellence Research Program on Healthy Aging (PIEI-ES).

Competing interests: The authors have declared that no competing interests exist.

P068-F | A translational, high-throughput proteomics platform for the deep analysis of plasma samples

E. Núñez*,†; E. Calvo*,†; E. Bonzón-Kulichenko*,†; R. Ponce*; F. García-Marqués*; M. Gómez-Serrano*; R. Campo*; R. Magni*; L. Fernández-Friera*,‡; A. Fernández-Ortiz*,§; B. Ibañez*,†,§; J. Vázquez*,†

*Fundación Centro Nacional De Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; †CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; ‡Hospital Universitario HM Montepríncipe, Boadilla del Monte, Spain; §Hospital Universitario Clínico San Carlos, Madrid, Spain; ¶IIS-Fundación Jiménez Díaz Hospital, Madrid, Spain

Background: Translational Proteomics for protein analysis in clinical samples should ideally be able to: (i) analyze large cohorts in a short period of time; (ii) quantify a high number of proteins per sample using hypothesis-free and non-targeted analysis; and (iii) incorporate robust statistical models for quantitative data analysis. In an effort to attain these goals, we report here a new, fast and hypothesis-free workflow for the analysis of hundreds of plasma samples that allows the accurate and reproducible quantification of more than one thousand proteins.

Material and methods: 620 plasma samples with/without plasma depletion (140/480), were trypsin-digested, and peptides were TMT-labelled (10-plex), separated into five fractions using in-cartridge high-pH reversed-phase

chromatography and analyzed by mass spectrometry. Protein quantitation was carried out using novel automated workflows based on the WSPP statistical model.

Results: We applied the described workflow for the analysis of 480 non-depleted plasma samples (65 TMT experiments) and obtained high reproducible results. We quantified 600 proteins per experiment in a fast analysis without peptide fractionation (6 hours per experiment, 2 weeks all). Using peptide fractionation, 1300 proteins were quantified (30 hours per experiment, 2 months). Protein yield was higher in depleted samples, but depletion introduced a bias in quantification that affected to about half of the plasma proteome, as revealed by hierarchical clustering analysis. Interestingly, we observed a high correlation between biochemical quantitation and mass spectrometry measurement for several proteins ($P < 1e\text{-}17$). Finally, we detected several proteins that were able to discriminate gender. These results highlights the robustness of the new method for the high-throughput quantitative analysis of the deep plasma proteome.

Conclusions: We have developed a new workflow that is able to analyze hundreds of plasma samples with a very deep proteome coverage in a routine bases, and that could facilitate and improve clinical proteomics discovery in human blood plasma.

HEPATO-GASTROENTEROLOGY/LIPIDS

P069-F | Life expectancy is unaffected by thyroid function parameters in euthyroid subjects: the PREVEND cohort study

R. Dullaart; L. van Tienhoven-Wind; E. Gruppen; W. Sluiter; S. Bakker

University Hospital of Groningen, Groningen, The Netherlands

Background: Thyroid function status may impact on a wide number of health issues including (subclinical) atherosclerosis, diabetes, stroke and cancer. However, the association of thyroid function status with life expectancy in euthyroid individuals is unknown. We prospectively determined relationships of TSH, free T4, free T3 and anti-thyroid peroxidase (TPO) autoantibodies with life expectancy.

Materials and methods: TSH, FT4, FT3, and anti-TPO autoantibodies were determined in 2431 euthyroid individuals (50·8 % women) who were followed for a median period of 13 (interquartile range 12·6–13·1) years (PREVEND cohort). 218 (9%) subjects died during follow-up. Differences in life expectancy according to thyroid function parameters and anti-TPO status were tested using the log-rank test with left and right censoring. Median life expectancy was adjusted for the life expectancy of individuals in the general Dutch population with the same age and sex (www.CBS.nl).

Results: Subjects who had died were older, more likely to be men, and were more frequently classified with cardiovascular risk factors. TSH, TSH, FT4, FT3 and anti-TPO autoantibody status were similar in subjects who had died compared to subjects who were alive at the end of follow-up ($P = 0{\cdot}23–0{\cdot}43$). Life expectancy was similar in the upper vs lower two tertiles of TSH, and the lower vs the upper two tertiles of FT4 and FT3, and did not differ according to anti-TPO autoantibody status ($P = 0{\cdot}81–0{\cdot}92$).

Conclusion: Low-normal thyroid function according to TSH, FT4, and FT3 tertiles, as well as anti-TPO status does not influence life expectancy in euthyroid individuals.

P070-F | Severe hyperlipidemia in chronic cholestatic liver disease treated by apheresis

V.F. Koehler; K.G. Parhofer

Medical Department IV, Campus Grosshadern, University Munich, Munich, Germany

Background: Cholestatic liver disease can lead to severe hypercholesterolemia. Hypercholesterolemia in intra- and extrahepatic cholestasis is characterized by the presence of lipoprotein-X (LpX). LpX is an abnormal low-density lipoprotein which in contrast with low-density lipoproteins (LDL) is non-atherogenic although LpX-cholesterol is usually measured as part of LDL-cholesterol.

Material and methods: We report on a 49 year old male patient with severe hypercholesterolemia and xanthoma striatum palm are presenting to our outpatient metabolic clinic in Munich, Germany. The lesions were painful and severely impacted the patient's everyday life (e.g. opening bottles, handshake). His past medical history included a 2-year history of secondary biliary cirrhosis following polytrauma and long-term ICU treatment (ischemic cholangiopathy) for which he was listed for liver transplantation.

Results: Biochemical evaluation revealed a total-cholesterol level of 970 mg/dL, triglyceride level of 158 mg/dL,

LDL-cholesterol level of 875 mg/dL with presence of LpX and high-density (HDL)-cholesterol level of 64 mg/dL.

Conclusions: Since lipid-lowering drugs were contraindicated, treatment with LDL-apheresis was started. Within 3 months of weekly LDL-apheresis, the lesions almost completely disappeared and the patient reported a significant relief in pain and function. After 1 year of treatment, the patient had a mean pre- to post-apheresis total-cholesterol reduction of 40% and LDL-cholesterol reduction of 36 %. Now 10 years later, the patient is in stable condition undergoing biweekly LDL-apheresis and not under consideration for liver transplantation. Pre-apheresis total-cholesterol level decreased to 220 mg/dL and LDL-cholesterol to 110 mg/dL.

P071-F | Activation of FGFR-signaling mediates resistance of gastrointestinal stromal tumors (GISTs) to imatinib (IM)

E. Garanina*; S. Boichuk†; P. Dunaev†; A. Galembikova†; E. Shagimardanova*; O. Gusev*; S. Khaiboullina*

Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation

Background: Secondary resistance to IM-based therapy in GISTs is the main cause of the tumor relapse. The gap in understanding of the molecular mechanisms regulating resistance to IM hampers the identification of novel molecular targets to restore GISTs sensitivity to anticancer therapeutics.

Material and methods: IM-resistant GIST T-1 subline (GIST T1-R) was established after a long-term culture with an increasing concentrations of IM. IM-naïve and resistant GISTs were cultured with IM, FGFR inhibitor BGJ398 alone or in combination. Secondary KIT mutations were analysed using the TruSight™ Cancer Sequencing Panel. The Proteome Profiler Human Phospho-Kinase Array Kit was used to measure protein phosphorylation in GIST cells. GISTs viability and growth kinetics were analyzed using MTS-based assay and iCELLigence system, respectively.

Results: GISTT-1R subline resistant to IM was established. IC50 for IM-naive GIST T-1 cell line was 1·19 ± 0·12 μM, whereas IC50 for GIST T-1R subline was 10 μM. Interestingly, the IM resistance was no associated with secondary KIT mutations, which were previously shown in IM-resistant GISTs. GIST T-1R was characterized by increased expression of pFGFR2α, whereas expression of pKIT and pPDGFRA was decreased. BGJ398-induced inhibition of FGFR reduced cellular viability, induced apoptosis and affected the growth kinetics of IM-resistant GISTs. In contrast, IM-naive GIST T-1 cells were not susceptible to FGFR inhibition. Importantly, inhibition of FGF-signaling restored the susceptibility to IM in IM-resistant GISTs.

Conclusion: The resistance to IM in GIST cells could be explained by the new type of RTK switch (loss of c-KIT/gain of FGFR2α). Our data highlights the potential therapeutic application of FGFR inhibitors for treatment of patients with IM-resistant and un-resectable forms of GISTs with the type of RTK switch indicated above.

This study was supported by a grant from the Russian Science Foundation (#14-15-00342). Work was also supported by Program of Competitive Growth of KFU.

P072-F | Glucose responsive polymeric nanocontainers for insulin delivery

I. Antipin*,†; A. Ziganshina*,†; T. Sergeeva†; R. Mukhitova†; I. Nizameev†; M. Kadirov†; A. Voloshina†; V. Zobov†

Kazan Federal University, Kazan, Russian Federation; †A.E. Arbuzov Institute of Organic and Physical Chemistry, Kazan, Russian Federation

Recently, the creation of the glucose responsive systems for the insulin delivery have been applied much attention due to the growing number of people with diabetes every year. The controllable release of insulin with increasing of glucose is necessary for the treatment of diabetes mellitus. We present a simple method for the synthesis of glucose responsive container for insulin delivery. The method is based on the self-assembly of sulfonated resorcinarenes (SRA) and phenyl boronic acid (BA). A mixing of water solutions of SRA and BA with insulin oil solution results in the microemulsion formation, where SRA and BA are located at the oil-water interface. The charged sulfonate groups of SRA are directed into the water while the tails in the low rim are pointed to BA in the oil phase. Upon standing of the microemulsion at room temperature at pH 8, the boronate ester bonds between SRA and BA are formed, which leads to the closed polymeric particles formation with encapsulated insulin.

The dissociation of the capsule and the insulin release occurs with an increase in glucose concentration. Excess glucose interacts with phenyl boronate destroying the bonds between SRA and BA. It leads to the decay of the capsule and insulin release.

Work supported by Program of Competitive Growth of KFU and by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7).

P074-F | Quality of life in patients with irritable bowel syndrome: the impact of a customized nursing intervention

M.V. Milaciu; M.-C. Mureşan; D. Sâmpelean; V. Negrean; L. Ciumărnean

"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-napoca, Romania

Background: The aim of this study was to evaluate the quality of life in patients with Irritable Bowel Syndrome in a hospital-setting and 1-month after applying a customized plan of nursing interventions.

Material and methods: The study was conducted during January 2017–April 2017 in the CF Clinical Hospital Cluj-Napoca, being previously approved by the hospital`s ethics committee. Fifty-two patients diagnosed with Irritable Bowel Syndrome were included in the study. We collected a full anamnesis for each patient, and they completed the SF-36 questionnaire (a validated tool for evaluating the general quality of life), the IBS-QoL and the Birmingham IBS questionnaires (which evaluate the quality of life in patients with irritable bowel syndrome). We applied a self-designed nursing intervention and we performed a 1-month phone visit to record the responses for the same questionnaires. We analyzed the results using Microsoft Office and an online descriptive statistics instrument.

Results: Mean age in our group was 58 years. Constipation was recorded as predominant symptom in 48% of the patients. We noted the results from the 3 questionnaires, collected within the index of hospitalization. We applied a nursing intervention combining 3 different non-pharmacological approaches, designed for patients from Romania. Each questionnaire showed improvement in the symptoms and quality of life of our patients after 1 month follow-up. There was a significant improvement in all health concepts of the SF-36 questionnaire ($P < 0{\cdot}01$). The IBS-QoL questionnaire has shown improvement of quality of life ($P < 0{\cdot}05$), except for the sexual-related issues scale. The total score of symptoms assessed by the Birmingham IBS questionnaire was significantly decreased after 1 month follow-up ($P < 0{\cdot}01$).

Conclusion: Our study showed that a customized plan of nursing intervention increased the quality of life in patients with Irritable Bowel Syndrome, 1-month after hospital discharge.

P075-F | The effects of myeloid cell-derived IL-1 alpha on acute colon inflammation

E. Voronov; M. Bersudsky; I. Kaplanov; R.N. Apte

Ben Gurion University, Beer Sheva, Israel

Background: The role of IL-1α in the development of IBD was shown by us. The redundant amounts of IL 1α have been found in WT mice, under homeostatic conditions and during colon inflammation, mainly in intestine epithelial cells (IECs). Previously, we found that IL-1α released from damaged colon tissue initiates and supports colon inflammation, induces recruitment of myeloid cells, which secrete other pro-inflammatory cytokines. Mice deficient in IL-1α presented a more moderate form of disease.

Methods: For the better understanding the effects of IL-1α in DSS-induced acute colon inflammation, we used mice with the specific deletion of IL-1α in IECs or myeloid cells.

Results: Mice deficient in IL-1α in IECs represent similar to complete IL-1α KO patterns of colon inflammation and were resistant to DSS-induced colitis and show less mortality. Mice with specific deletion of IL-1α in myeloid cells showed a more severe form of the disease and higher mortality compared to control mice. The increased influx of T cells, especially CD8 and Treg-positive cells was correlated with deficiency of IL-1α in IECs and good prognosis of disease. These cells are known to be important in the keeping of the colon homeostasis and previously were shown to be correlated with good prognosis. In IL-1α deficient in myeloid cells mice, the amount of these cells was decreased. In these mice, we also observed an increased amount of pro-inflammatory monocytes and decreased levels of neutrophils in inflamed colon.

Conclusions: Our results show that IL-1α from colon epithelial cells is an important molecule in the progression of colon inflammation, whereas IL-1α from myeloid cells is sufficient for colon protection. The mechanisms involved in effects of IL-1α from myeloid compartment in the development of acute colon inflammation will be discussed.

P076-F | Novel molecular mechanisms affecting sensitivity of imatinib (IM)-resistant gastrointestinal stromal tumors to the topoisomerase type II inhibitors

E. Garanina*; S. Boichuk†; A. Galembikova†; P. Dunaev†; S. Khaiboullina*

**Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation*

Background: The acquired resistance of gastrointestinal stromal tumors (GISTs) to the targeted-based therapy remains the driving force to find out the novel approaches that are capable to increase the sensitivity of GISTs to the current therapeutic regimens. Despite the well-known historic evidence that GISTs are resistant to conventional chemotherapy, newer studies – including our own – have reported that GIST cells are sensitive to the certain chemotherapeutic drugs, e.g. the topoisomerase II inhibitors.

Material and methods: IM-naïve and resistant GISTs were cultured with doxorubicin or etoposide, FGFR inhibitor BGJ398 alone or in combination. GISTs viability and growth kinetics were analyzed using MTS-based assay and iCELLigence system, respectively. Protein expression was examined by western blotting.

Results: BGJ398, a selective FGFR inhibitor, sensitized imatinib (IM)-resistant GIST cells with RTK switch (loss of c-KIT/gain of pFGFR2a) to the low doses of topoisomerase type II inhibitors – doxorubicin and etoposide. This was due to substantial decrease of Rad51 recombinase expression and inhibition of homologous recombination (HR)-mediated DNA repair. Similar results were obtained when an overexpressed pFGFR2a was knocked down in GIST T-1R cells by corresponding siRNA prior the chemotherapeutic drugs exposure.

Conclusion: Our data illustrates that combined therapy (RTKs inhibitors supplemented with low-doses of topoisomerase type II inhibitors) might be effective for the patients with unresectable and metastatic forms of GISTs. FGFR inhibitors might have a perspective use due to their ability to sensitize IM-resistant GISTs to topoisomerase type II inhibitors and induce tumor cell apoptosis via targeting DNA DSBs repair mechanisms.

This study was supported by a grant from the Russian Science Foundation (Grant No. 14-15-00342). Work was also supported by Program of Competitive Growth of KFU.

P077-F | The burden of abdominal surgery in patients with Familial Mediterranean Fever (FMF) in Apulia and Basilicata, southern Italy

P. Portincasa*; G. Calamita†; G. Scaccianoce‡

**Department of Biomedical Sciences & Human Oncology, University "Aldo Moro" of Bari Medical School, Bari, Italy; †Department of Biosciences, Biotechnologies and Biopharmaceutics, University "Aldo Moro", Bari, Italy; ‡Gastrointestinal Endoscopy, "Umberto I" Hospital, Altamura, Bari, Italy*

Background: FMF is a hereditary autosomal recessive autoinflammatory disorder caused by MEFV gene Chr 16p13.3 encoding the protein pyrin (marenostrin), 781 amino acids and expressed predominantly in the cytoplasm in cells of myeloid lineage. We recently identified a novel cluster of italian FMF patients (Bonfrate L. et al., Eur J Clin Invest 2017; 47: 622–9.) reporting an instructive surgical history.

Material and methods: 49 FMF subjects (M:F = 26:23, age 38 years ± 2 SE) were identified between Matera (Basilicata Region) and Altamura (Apulia Region) and observed for up to 8 years. FMF diagnosis was confirmed by genetic analyses.

Results: Age at disease onset was 22·1 years ± 1·2 SE (diagnostic delay 15·5 years ± 1·9 years); 82% of patients suffered from abdominal pain, and serum amyloid A was increased in 20% of patients. A prior abdominal surgery was recorded in 17/49 (35%) patients: appendectomy (65%), laparotomy (24%) and cholecystectomy (13%), but none of the surgical interventions was associated with symptom improvement. The surgical group was older (43·3 years ± 4·4 years vs 28·6 ± 4·6 years, $P = 0{\cdot}03$), had greater International Severity Scoring System (ISSF) (3·5 ± 0·4 vs 2·5 ± 0·2, $P = 0{\cdot}02$), and longer diagnostic delay (24·4 ± 5·2 vs 12·2 ± 1·7 years, $P < 0{\cdot}01$) than the surgery-free group. The variant c.2080A>G (M694V, exon 10) was present in heterozygosity in 37% of surgical group (vs 0% among the surgery-free group). In over 98% of patients, inflammation markers, duration and intensity of febrile painful attacks, quality of life and ISSF score improved dramatically following colchicine treatment (Acarpia Farmaceutici SrL, Italia). Emergency abdominal surgery could be avoided in one female FMF patient, when surgeons were advised.

Conclusions: Apulia and Basilicata regions are new endemic areas for FMF. Poor awareness of FMF exposes the patients to the risk of unnecessary surgeries, post-surgical morbidity and complications. Educational programs are being promoted to reduce the burden of FMF disease.

P078-F | Athletes have longer telomeres than age-matched controls

E. Valeeva*,†; I. Ahmetov†; O. Kravtsova*

**Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation*

Introduction: The length of telomeres is an indicator of the mitotic activity of our cells. There are many external and internal factors that have favorable effects on the telomere length. It is well established that physical activity favorably affects the length of telomeres.

Aim: To assess the effect of physical activity on the relative telomere length (RTL) in athletes and non-athletes depending on the age.

Materials and methods: The RTL of telomeres was measured by quantitative real-time PCR in Russian athletes (N = 895, aged from 8 to 77 years, 72% and 28% male and female respectively) and non-athletes group (N = 508, aged from 7 to 72 years, 43% and 57% female respectively). All participants were divided into 5 groups: aged from 7 to 11 years (78 athletes vs 15 non-athletes), II – 12–20 years. (N = 503 vs N = 209, respectively), III – 21–45 years (N = 291 vs N = 205), IV – 46–60 years (N = 19 vs N = 73), V – above 60 years (N = 4 vs N = 6). RTL was calculated according to the original formula of the author Cawthon (2002). Statistical analysis was processed using GraphPad InState.

Results: The difference of average RTL between athletes (1·190 ± 0·094) and non-athletes (1·172 ± 0·134) was significant (P = 0·004). Further, our study samples were compared by age groups: the athletes of group I (1·167 ± 0·102 vs 1·179 ± 0·125, P = 0·003), II (1·188 ± 0·086 vs 1·146 ± 0·158, P < 0·001) and group III (1·196 ± 0·103 vs 1·177 ± 0·091, P = 0·03) had significantly higher RTL than non-athletes. Group IV and V didn't differ significantly from each other (P > 0·05). Correlation between RTL and age was significant for both athletes (R = 0·13, P = 0·001) and non-athletes (R = 0·26, P < 0·001).

Conclusion: Our data indicate that athletes have longer telomeres than age-matched controls, probably due to their increased physical activity.

This work was supported by Program of Competitive Growth of KFU.

P079-F | The interrelationship between relative telomere length and sports success

E. Valeeva*,†; I. Ahmetov†; O. Kravtsova*

*Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation

It has been established that telomere length positively correlates with the level of physical activity, thus indicating that movement prolongs a person's life years. The purpose of this study was to study the association of relative telomere length (RTL) with physical activity and athletic success.

The study involved 921 Russian athletes (71% men, 20·7 ± 8·4 years, power athletes [n = 274], long-distance athletes [n = 58], middle-distance athletes [n = 186], combat athletes [n = 108], athletes with mixed activity [n = 295]) and 689 non-athlete (36% men, 20·8 ± 16·5 years). RTL was calculated according to the original formula of the author Cawthon (2002). Statistical analysis was processed using GraphPad InState.

On average, the RTL of all athletes was 1·187 ± 0·098 vs 1·176 ± 0·132 controls (P = 0·049). The longest telomeres had power athletes (1·197 ± 0·102), then combat athletes (1·192 ± 0·094), middle-distance athletes (1·964 ± 0·086), long-distance athletes (1·177 ± 0·118) and the shortest telomeres had athletes with mixed activity (1·172 ± 0·101). Correlation analysis showed that with the growth of athletic achievement, the lengths of telomeres of athletes increase (P < 0·0001, r = 0·15, Honored Master of Sports (n = 3) – 1·225 ± 0·075, Master of Sports of International Class + Master of Sport (n = 184) – 1·206 ± 0·095, Candidate Master of Sports (n = 263) – 1·192 ± 0·101, recreational athletes (n = 174) – 1·192 ± 0·098, non-elite athletes (n = 310) – 1·169 ± 0·094).

Our findings show that athletes have significantly longer telomeres than controls, which may be due to their increased level of physical activity. In addition, it was also established that elite athletes had longer telomeres than less successful athletes.

This work was supported by Program of Competitive Growth of KFU.

P080-F | Eradication of *Helicobacter pylori* infection in Apulia: let's do it better

M. Lorusso*; M. Domenica di Palo*; O. de Bari*; P. Vitellio*,†; R. Monaco*; D. De Vito†; P. Portincasa*

*Clinica Medica "A. Murri", Department of Biomedical Sciences and Human Oncology, University of Bari "A. Moro", Bari, Italy; †Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari "A. Moro", Bari, Italy

Background: *H. pylori* (HP) chronic gastritis is the commonest bacterial infections worldwide, causing symptoms and risk to develop peptic ulcer disease, atrophic gastritis, metaplasia, gastric cancer and mucosa-associated MALT lymphoma. Guidelines suggest HP eradication in HP(+)ve subjects. In clinical practice, access to several eradication regimens may confuses and detach from guidelines. We investigated the "real life" scenario about noninvasive diagnosis (urea breath test, UBT) and ongoing HP eradication.

Methods: 3418 subjects with suspected HP infection were referred for UBT to a 3rd referral center in southern Italy. HP infection rate, treatment regimens and eradication rates were recorded.

Results: 1433 HP(+)ve patients underwent eradication therapy which succeeded in 1114 (78%) patients. Specific eradication rates were: 97% Pylera® (PPI + bismuth subcitrate potassium + metronidazole + tetracycline, N = 201), 84%

Sequential (PPI + amoxi 1 g for 5 days then PPI + clarithro 0·5 g + tinidazole 0·5 g for 5 days, $N = 294$); 65% Triple (PPI + clarithro 0·5 g + amoxi 1 g for 7 days, $N = 121$), 57% Levo (PPI + amoxi 1 g + levofloxacin 0·25 g for 10 days, $N = 24$), 100% Concomitant (PPI + clarithro 0·5 g + amoxi 1 g for 14 days, $N = 2$) and 73% Undefined (likely Triple: patients missing documentation, $N = 470$). In general practice, Triple and "Undefined" were the most popular regimens as 1st (96%) to 5th line treatments. Referral center used 1st line Sequential/Concomitant and 2nd line Levo until 2016 and then 1st/2nd line Concomitant & lt; Pylera®.

Conclusions: In geographical regions with high clarithromycin resistance, the use of Triple/Sequential/Undefined eradication regimens for HP infection does not follow current guidelines. This approach causes ineffective, harmful antibiotic (over)-prescription and potential intestinal dysbiosis. A better training program is necessary to avoid unnecessary over- misuse of antibiotics for HP eradication, especially in General Practice.

P081-F | Peculiarities in nutrition of the adult population of the city of Kazan

S. Fomina; N. Stepanova
Kazan Federal University (KFU), Kazan, Russian Federation

Background: At present, significant changes in the structure and quality of nutrition of the population living in the territory of the Russian Federation take place. Incompetence in food culture and unhealthy eating becomes a serious risk factor for many diseases.

Material and methods: A survey questionnaire of the population of the city of Kazan (256 persons) aged from 18 years and older; analysis of the morbidity was carried out based on the annual reports (form No. 12) from the medical institutions of Kazan (2005–2016).

Results: The study of nutrition showed that 55% took meals irregularly and monotonously. Every 6th–7th respondent took meals once a day. The frequency of taking meat products 1–2 times a week among the surveyed made 27·6%. The population preferred fish and sea products once (58·5%) or two-three (27·2%) times a week. 29% of the surveyed population included cereals into the menu 2–3 times a week, and 27·7% – once a week. Our study revealed that 2·1% of the population never ate vegetables and fruit, 14·3% – once and 27·3% 2–3 times a week.

Conclusions: Analysis of the morbidity showed that the highest growth rates of the primary disease incidence among the adult population compared with the year of 2005 was determined in the class of K00-K92 – 36% (1st place), G00-G98 – 7·3% (2nd place), I00-I99 – 3·2% (3rd place). The primary incidence of digestive diseases increased significantly for 10 years ($R^2 = 0·6943$). Thus, imbalanced nutrition has a considerable effect on the incidence level of digestive diseases.

This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities 19.9777.2017/8.9

P082-F | Antibiotic resistance of dairy and probiotic lactobacilli and its transfer to pathogenic bacteria

E. Anisimova; N. Bruslik; D. Yarullina
Kazan Federal University, Kazan, Russian Federation

Background: Lactobacilli are common in foods and are members of the resident gastrointestinal microbiota of humans. These bacteria may function as hosts of antibiotic resistance genes, which can be transferred to pathogenic bacteria. The aim was to characterize antibiotic resistance of Lactobacillus spp. and to estimate the potential transfer of resistance genes from lactobacilli to opportunistic pathogens that share the same intestinal habitat.

Methods: Nineteen Lactobacillus strains were isolated from probiotics and dairy products and identified by MALDI Biotyper. They were screened for phenotypic resistances to 14 antibiotics by the disk diffusion method. The erythromycin (Erm) and tetracycline (Tet) resistance genes were amplified by PCR and sequenced (Sanger method). The tet(K) gene from plasmid DNA of *L. fermentum* strain 5–1 was transferred by electroporation to sensitive bacteria Citrobacter freundii, which became resistant to Tet, as proved by growth on agar with Tet and PCR amplification of tet(K) gene.

Results: *L. plantarum* and *L. fermentum* isolates showed the resistance profile characteristic for lactobacilli. They possessed intrinsic resistance to ciprofloxacin (84·2% of strains) and vancomycin (68·4% of strains), while showing susceptibility to protein synthesis inhibitors, except aminoglycosides. Most strains were susceptible to beta-lactam antibiotics and rifampicin.

The Erm resistance gene erm(B) was detected in chromosomal DNA of *L. fermentum* 5–1, corresponding to its resistance phenotype. These strain sensitive to Tet was positive for silent genes tet(K) and tet(M) in plasmid DNA. Moreover, tet(K) gene of *L. fermentum* 5–1 was successfully transferred by electroporation to sensitive bacteria *Citrobacter freundii*, which became resistant to Tet.

Conclusions: This study presents a cause for concern because dairy Lactobacillus strain has been demonstrated to serve as reservoir organism for acquired resistance genes that can be spread to pathogenic bacteria.

This work was supported by Program of Competitive Growth of KFU and RFBR grant No 17-00-00456 and 18-34-00268.

P083-F | Effects of the lactobacilli supplementation on the anxious-phobic state of mice with altered microbiota

A. Arslanova; M. Rudich; K. Chernova; O. Yakovleva

Kazan (volga Region) Federal University, Kazan, Russian Federation

Intestinal microbiota is a bacteria community that helps to maintain a dynamic metabolic balance in the body. The microflora can affect the physiological, behavioral and cognitive functions of the brain. This two-way communication system forms the axis "microbiota-intestine-brain." Therefore, changes in the intestinal microbiota can affect the functions of the digestive system and the CNS. Changes in microflora in cases of infection, disease and a wrong diet can lead to a dysbacteriosis that causes anxiety and stress. The purpose of this study was to compare the anxious-phobic state of mice in control and with altered microbiota.

Experiments were performed on mice aged 20–25 days. Animals were divided into 3 groups: (i) a control group receiving i.p. injections of saline (n = 10); (ii) mice receiving i.p. injections of antibiotics (a cocktail of neomycin, vancomycin, amphotericin B, ampicillin, metronidazole, AB, n = 10); (iii) mice receiving injections of antibiotics together with supplementation of lactobacilli to their water (4×10^6 cells/mL, AB + LB, n = 10). To assess the anxiety state an Open Field, the Black/White Camera test and an integral anxiety index (IAI) were used.

In the control animals no changes were observed in all tests. IAI was slightly decreased reflected the gradual adaptation of animals. AB group demonstrated the rise of emotionality: an increase in the number of acts of grooming and defecation, a decreased time of leaving from the center compared with the control and AB + LB groups. The animals of AB group demonstrated the reduced time spent in the light chamber and an increase of IAI, whereas in AB+LB group the time spent in the light chamber and IAI didn't change.

Thus, the disturbance of normal microflora leads to the anxious-phobic state in mice. Simultaneous supplementation of lactobacilli prevented the observed changes, which indicate a positive effect of normal microflora on stress resistance.

P084-F | The effects of short-chain fatty acids on the motility of the mouse colon

I. Shaidullov; D. Sorokina; K. Chernova; M. Shafigullin; G. Sitdikova

Kazan (volga Region) Federal University, Kazan, Russian Federation

Short chain fatty acids (SCFAs), such as acetate, propionate, and butyrate are key products' of fermentation of indigestible carbohydrates by commensal bacteria that reside in the gastrointestinal tract. SCFAs used as a nutrient source by colonic epithelial cells and induced apoptosis in colon cancer cell lines. The colonic SCFAs content may change in patients with irritable bowel syndrome and inflammatory bowel diseases compared with healthy controls. It has been shown that, besides their effect on gut morphology and function, SCFAs have excitatory or inhibitory effects on gastrointestinal motility. However, the mechanisms of SCFAs action on colonic motility are not completely elucidated. In this study, we investigated SCFAs effects on spontaneous and carbachol-induced contractions in mouse colon.

We recorded the contraction of mouse colon segments of 5 mm length under isometric conditions. During the experiment, the organ bath was filled with 37°C Krebs solution continuously bubbled with carbogen. Carbachol was used in concentration 1 μM.

After a stable baseline was attained acetate, propionate or butyrate in concentration range from 0·5 to 10 mM were added cumulatively to the bath. All SCFAs induced dose-dependent decrease of the contraction frequency. Acetate and propionate decreased tonic tension without effect on the amplitude of phasic contraction; butyrate decreased the amplitude of the phasic contraction without effect on the tonic tension. Preliminary application of SCFAs at concentration 10 mM decreased the amplitude of carbachol induced increase of the tonic tension.

In summary, this study shows that short chain fatty acids induced dose-dependent inhibitory effects on spontaneous and carbachol induced contraction of the colon. It was concluded that SCFAs may impact in alteration of colonic motility during inflammatory bowel disease and irritable bowel syndrome. Work supported by Program of Competitive Growth of KFU.

P085-F | Antioxidative and anti-inflammatory effects in the realization of hepatoprotective properties of derivative of drug Xymedon with L-ascorbic acid

A. Vyshtakalyuk*; R. Diabankana†; A. Parfenov*; L. Gumarova*; T. Povysheva*; V. Zobov*; I. Galyametdinova*; V. Semenov*

*A.E. Arbuzov Institute of Organic and Physical Chemistry – Subdivision of the Federal State Budgetary Institution of Science "Kazan Scientific Center of Russian Academy of Sciences", Kazan, Russian Federation; †Kazan Federal University, Kazan, Russian Federation

The inflammation and activation of peroxide oxidation of lipids are important components in the pathogenesis of liver diseases. In our previous investigations was shown that pyrimidine derivative, Russian drug Xymedon (I) have hepatoprotective properties [1]. We synthesized derivatives of Xymedon with biogenic molecules to improvement of hepatoprotective properties [2,3]. The derivative of Xymedon with L-ascorbic acid (II) leads to the most pronounced decrease of areas of liver injury [3,4]. However, the mechanism of hepatoprotective action of (II) didn't studied.

In present work we studied expression of superoxide dismutase (SOD1) and glutathione peroxidase (ISO1) as parameters of antioxidative mechanism and cytokines level as parameters of anti-inflammatory mechanism. Parameters were investigated in liver lysates obtained from rats that have been subjected to toxic damage by CCl_4 and treated with (II). SOD1 and ISO1 were determined by immunoblotting method, cytokines – by multiplex analyzer MagPix and Merck kits.

After administration of CCl_4 the SOD1 expression was increased in 10 times, ISO1 was decreased in 2·7 times. On the 3rd and 21st day the SOD1 level in control was down but stayed higher then initial one in 2 times. ISO1 remained at a reduced level. The elevated level of SOD1 in groups injected with (II) stayed up to 21st day. (II) was no effect on ISO1. In control, cytokines IL-1α, IL-3 and IFNγ were increased after CCl_4 administration and remained at an elevated level for 3 days. The level of proinflammatory cytokines IL1-α, IL1-β in groups treated with (II) was lower than in control.

References: 1. Vyshtakalyuk AB, et al. Doklady Biochemistry and Biophysics. 2015. 462:143–146.

2. Litvinov IA, et al. J. Struct. Chem. 2016. 57:549–556.

3. Vyshtakalyuk AB, et al. Russian Journal of Bioorganic Chemistry. 2017. 43(5):604–611.

4. Vyshtakalyuk A, et al. BioNanoScience. 2017. 7(4):616–622.

The research was financed by Russian Science Foundation grant number 14-50-00014.

P086-F | Gut microbiota in chronic constipation disease: a new therapeutic target

A.A. Arzamasceva*; A.S. Volkova*; M.U. Shafigullin*; D.R. Khusnutdinova*; E.A. Anisimova*; K.A. Sakulin†; O.J. Karpukhin†; G.F. Sitdikova*; D.R. Yarullina*

*Kazan Federal University, Kazan, Russian Federation; †Kazan State Medical University, Kazan, Russian Federation

Background: Chronic constipation (CC) is a prevalent, burdensome gastrointestinal disorder whose etiology remains poorly understood and is most likely multifactorial with most significant contribution of imbalance in intestinal microbiota and failure of gut motor function. We have recently shown endotoxemia and morphological pathology in the colonic wall in constipated patients. The aim of this study was to characterize mucosal microbiota and contractility of colonic muscle in CC patients.

Methods: Colonic tissue samples were obtained from patients undergoing colectomy for CC and contractile activity was analyzed. Outcome was compared with the intestinal muscle contractions of patients undergoing colorectal surgery for gut diseases not associated with disorder of motor function. The juxta-mucosal microbiota was studied with culture-based and 16S rRNA pyrosequencing techniques.

Results: In CC patients, the spontaneous contractions were higher in both longitudinal (12·3 ± 4·4 vs 1·9 ± 0·9 g/s) and circular (13·47 ± 3·3 vs 5·8 ± 2·2 g/s) smooth muscle strips compared to controls. Moreover, the carbachol-induced response was also increased in CC in both longitudinal (EC50 0·50 ± 0·05 vs 0·65 ± 0·14 μM) and circular (EC50 0·76 ± 0·06 vs 2·01 ± 1·05 μM) muscle layers compared with those of the control group. The microbiota in constipated patients was dominated by bacteria belonging to the phyla Firmicutes (31–68%) and Bacteroidetes (5–57%), followed by Proteobacteria (1–48%) and Actinobacteria (1–17%). No definitive association between constipation and the abundance or lack of certain prokaryotic taxa in the gut microbiome was observed. Yet, we identified some microbes which may affect motility via production of methane (Methanobrevibacter), hydrogen sulfide (Desulfovibrio, Bilophila), butyrate (Clostridiales), propionate (Bacteroides), and acetate (many taxa).

Conclusions: Our findings suggest that alterations of the microbiota might affect gut motility via altered microbial-derived metabolites, and the restoration of disturbed microbiota may be a novel therapy strategy for CC.

This work was supported by Program of Competitive Growth of KFU.

P087-F | Detailed histopathological analysis of Caroli's syndrome case

M. Mavlikeev*; A. Titova*; A. Chebochakova*; I. Plaksa[†,‡]; N. Gizatullina[§,¶]; A. Kiyasov*; R. Deev[‡,**]

*Kazan Federal University, Kazan, Russian Federation; [†]Moscow City Oncological Hospital No. 62, Moscow, Russian Federation; [‡]Institute of Human Stem Cells, Moscow, Russian Federation; [§]Interregional Clinical Diagnostic Center, Kazan, Russian Federation; [¶]Kazan State Medical University, Kazan, Russian Federation; **Ryazan State Medical University named after academician I.P. Pavlov, Ryazan, Russian Federation

Caroli syndrome is a rare (frequency 1:1 000 000) autosomal recessive hereditary disease characterized by a cystic dilatation of the intrahepatic bile ducts in combination with portal fibrosis leading to portal hypertension. This disease is caused by a mutation in the PKHD1 gene coding fibrocystin – a component of the primary cilia of the epithelium, as a result of which the formation of bile ducts is disrupted. We described a clinical case with detailed histopathological analysis.

Samples of internal organs of the male patient who died at the age of 37 because of dysmetabolic encephalopathy were fixed in 10% neutral formalin and embedded in paraffin. Histological sections were stained with hematoxylin and eosin, Mallory trichrome, alcian blue, impregnated with silver by Foot, and immunohistochemically with antibodies against cytokeratins (CK) 7, 8, 19, CD4, CD8, MHCII, PCNA.

Histopathological analysis of liver sections showed wide layers of fibrous tissue with pseudonodes of various shapes and sizes. In the fibrous fields an increased number of bile ducts of different size was found. Ducts were dilated with wall ruptures, had a bizarre shape, contained gallstones and pus. The lining of the ducts was either pseudo-layered with hyperplastic proliferating cholangiocytes hypersecreting acidic mucopolysaccharides or atrophic. Lymphohistiocytic infiltration of ducts was observed. Fibrous fields and cyst walls of various sizes and shapes are infiltrated with $CD4^+$ lymphocytes which might be due to MHCII overexpression found in cholangiocytes. $CK7^+$ hepatocytes were also found.

Thus, important pathomorphogenetic components of the Caroli syndrome are severe portal fibrosis with extensive lymphocytic infiltration, a pronounced ductular reaction with dysplasia of cholangiocytes and a violation of the differentiation of the common precursor cell of cholangiocytes and hepatocytes. Work was supported by Program of Competitive Growth of KFU

P090-F | Influence of derivative of drug Xymedon with L-ascorbic acid on the cell composition of liver in rats with non-alcoholic steatohepatitis

A. Vyshtakalyuk*; L. Hasanshina[†]; L. Gumarova*; A. Parfenov*; G. Belyaev[†]; D. Kondrashina*; V. Zobov*; I. Galyametdinova*; V. Semenov*

*A.E. Arbuzov Institute of Organic and Physical Chemistry - Subdivision of the Federal State Budgetary Institution of Science "Kazan Scientific Center of Russian Academy of Sciences", Kazan, Russian Federation; [†]Kazan Federal University, Kazan, Russian Federation

In our previous work [1] the hepatoprotective properties of the drug Xymedon and its derivatives [2] was shown. Moreover, the ability of pyrimidine derivatives to stimulating the regeneration of the spinal marrow [3] as well as the blastema of planarian [4,5] have been determined.

The main goal of this work was to study the effectiveness of the derivative (II) of Xymedon with L-ascorbic acid on the model of non-alcoholic steatohepatitis in rats. To modelling the non-alcoholic steatohepatitis, Sprague Dawley rats 5–6 month age were kept on the high fat (10%) ration with addition 2% of cholesterol during 30 days. After that, we administered orally (II) in the dose 3·4 mg/kg, Xymedon (I) and L-ascorbic acid (III) in equivalent doses 1·7 and 1·8 mg/kg respectively during 28 days. After euthanasia of rats, liver samples were processed in the histoprocessor Sacura Tissue TekVip.5, sections of liver were stained with hematoxylin-eosin and Sudan, then studied according to the method [6].

Areas of fat detection in liver were reduced in groups treated with (I), (II) and (III) ($1{\cdot}5 \pm 0{\cdot}2$; $7{\cdot}4 \pm 1{\cdot}0$ and $5{\cdot}6 \pm 0{\cdot}8$% respectively) in contrary to control ($20{\cdot}0 \pm 2{\cdot}0$%). The total number and number of binuclear hepatocytes on the unit of square of liver was more, while the number of hepatocytes with fatty, balloon, hydropic dystrophy and necrosis was less in experimental groups in comparison to the control. (II) is effective against non-alcoholic steatohepatitis.

References:

1. Vyshtakalyuk AB, et al. Doklady Biochemistry and Biophysics. 2015. 462:143–146.
2. Vyshtakalyuk AB, et al. Russian Journal of Bioorganic Chemistry. 2017. 43(5):604–611.
3. Povysheva T.V., et al. Bulletin of Experimental Biology and Medicine. 2016. 162(2):220–224
4. Porfiriev A.G., et al. BioNanoScience. 2017. 7(1):237–239.
5. Porfiriev A.G., et al. BioNanoScience. 2017. 7(4):570–573.
6. Vyshtakalyuk A, et al. BioNanoScience. 2017. 7(4):616–622.

The research was financed by Russian Science Foundation grant number 14-50-00014.

OMICS

P129-T | GlycA, a novel pro-inflammatory glycoprotein biomarker, and high sensitivity C-reactive protein are inversely associated with sodium intake after controlling for adiposity: PREVEND study

R. Dullaart; E. Gruppen; M. Connelly; J. Otvos; S. Bakker
University Hospital Groningen, Groningen, Netherlands

Background: The extent to which dietary sodium intake may confer alterations in inflammatory status is unclear. GlycA is a novel pro-inflammatory proton nuclear magnetic resonance spectroscopy biomarker which associates with the development of cardiovascular disease and diabetes. We determined associations of the inflammatory markers GlycA and high sensitivity C-reactive protein (hsCRP) with 24-hour sodium excretion.

Materials and methods: A cross-sectional population-based study was performed among 3935 subjects, not using anti-hypertensive medication, lipid lowering drugs or glucose lowering treatment. Urinary sodium excretion was calculated as the mean of two 24-hour urine excretions. Linear regression models were used with 24-hour sodium excretion as an independent variable and GlycA or Loge hsCRP as dependent variables.

Results: Mean sodium excretion was 143·0 ± 53·4 mmol/24-hour. GlycA was 343·6 ± 58·7 μmol/L and hsCRP (geometric mean, 95% CI) was 1·20 (1·16, 1·25) mg/L, respectively. In age- and sex-adjusted analyses, GlycA and Loge hsCRP were not significantly associated with 24-hour sodium excretion (B: 1·23 (95% CI: −0·67, 3·13), $P = 0·21$ and 0·03 (95% CI: −0·004, 0·07), $P = 0·08$, respectively per 1 SD increase). After additional adjustment for body mass index (BMI), both GlycA (B: −2·76 (95% CI: −4·65, −0·86), $P = 0·004$) and Loge hsCRP (B: −0·07 (95% CI: −0·11, −0·04), $P < 0·001$) were inversely associated with 24-hour sodium excretion. These associations were similar if adjustment was performed for waist circumference instead of BMI, or if additional adjustment was performed for relevant clinical and laboratory variables, and were particularly present in men.

Conclusion: The pro-inflammatory biomarkers, GlycA and hsCRP, are inversely related to higher 24-hour sodium excretion when taking into account variation in adiposity. These inverse relationships remain present after taking account of other covariates.

P130-T | High fat diet increases ATP levels but reduces glucose-derived Krebs cycle turn-over in the myocardium

I. Barba*,†; D. Aluja*,†; J. Inserte*,†; D. Garcia-Dorado*,†
**Vall d'Hebron University Hospital and Research Institute, Barcelona, Spain; †CIBER Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain*

Background: The obesity paradox holds that obesity may, be protective against ischemia/reperfusion injury. Although the effects of the obesity paradox have been shown in experimental models as well as in epidemiological studies, the end-effector mechanisms leading to the protection against ischemia/reperfusion are not fully understood. The objectives of the present work were to investigate if high fat diet would induce changes in the myocardial metabolic profile.

Material and methods: Twelve male mice were fed for 4 months with control diet ($n = 6$) or high fat diet ($n = 6$). After 2 months of feeding mice were injected with 1-13C-Glucose (200 mg/kg) i.p. for 10 minutes. After that, the hearts rapidly excised and frozen in liquid nitrogen. Metabolites were extracted using methanol:chloroform. 1H-NMR spectra were used to evaluate steady-state metabolism while 1H-13C HSQC was used to follow the fate of the labeled glucose.

Results: High fat diet induced an increase in ATP (35·22 ± 8·3 vs 22·58 ± 6·59 μmols/gr wet weight; $P = 0·02$) and alanine (65·17 ± 12·12 vs 39·53·58 ± 12·11 μmols/gr wet weight; $P = 0·002$). Other metabolites including creatine, glucose, glutamate, succinate and taurine did not change with diet.

1-13C-Glucose label fate showed a reduced level of glutamate in HFD mice (0·51 ± 0·21 vs 0·99 ± 0·21 $P = 0·03$). There were no differences in glucose, glucose-6P, and lactate. The reduction in glutamate label but not total metabolite pool suggests that the turn-over of glucose in the Krebs cycle is reduced in high fat diet fed mice in comparison to control diet.

Conclusion: High fat diet fed mice hearts have higher levels of ATP than control fed mice. Increased levels of Alanine together with reduced glucose-derived Krebs cycle rate is consistent with increased aerobic glycolysis. This has been shown to be protective and could explain, at least in part, the obesity paradox.

P131-T | Impact of phenol-enriched virgin olive oils on the circulating levels of microRNAs related to cardiovascular disease

L. Daimiel*; V. Micó*; M.J. Motilva†; L. Rubió†; M. Fitó‡,§; M. Farrás‡,§; M.I. Covas‡,§; R.M. Valls¶; A. Pedret¶; R. Solá¶; J.M. Ordovás*,**

*Nutritional Genetics and Epigenetics Group, Instituto Madrileño de Estudios Avanzados (IMDEA) Food Institute, CEI UAM+CSIC, Madrid, Spain; †Food Technology Department, Agrotecnio Center, Escola Tècnica Superior d'Enginyeria Agrària, University of Lleida, Lleida, Spain; ‡Cardiovascular Risk and Nutrition (Regicor Study Group), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain; §CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Institute of Health Carlos III, Spain; ¶Unit of Lipids and Arteriosclerosis Research, CIBERDEM, Hospital Universitari Sant Joan, IISPV, Universitat Rovira i Virgili, Technological Centre of Nutrition and Health (CTNS), Functional Nutrition, Oxidation and Cardiovascular Disease Research Group, Medicine and Surgery Department, Universitat Rovira i Virgili, Reus, Spain; **Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University School of Medicine, Boston, USA*

Background: The cardiovascular benefits of the Mediterranean Diet (MD) have been recently described. Extra virgin olive oil (EVOO) is the main source of fat in the MD and it has been suggested that EVOO is the component of the MD that most contributes to its cardioprotective effects probably mediated by its polyphenolic composition and its content of oleic acid. However, the mechanisms underlying the effect of EVOO consumption are not fully understood. microRNAs are well-known modulators of gene expression and recent studies have highlighted their potential as therapeutic tools. Moreover, microRNAs can circulate in plasma and are potential biomarkers of disease. We aimed to define if EVOO consumption with different levels of polyphenols modifies circulating levels of microRNAs related to cardiovascular health.

Material and methods: We analyzed a selected panel of 62 microRNAs in plasma samples of the VIRGIN OLIVE OIL AND HDL FUNCTIONALITY (VOHF) study corresponding to a postprandial intervention in 12 healthy individuals with EVOO enriched with its own polyphenol extract by different amount: L-FVOO with a low phenolic content (250 mg total phenols/kg of oil), M-FVOO with a medium phenolic content (500 mg total phenols/kg of oil) and H-FVOO with a high phenolic content (750 mg total phenols/kg of oil). microRNAs levels were analyzed by quantitative Real-Time PCR.

Results: L-EVOO intake modulated the expression of miR-10b, miR-221 and miR-125a-5p. M-EVOO modulated the expression of 12 microRNAs with some members of the miR-17-92 cluster, miR-192, miR-21 and miR-423-5p being up-regulated, whereas miR-223, miR-122 and miR-30c were down-regulated. H-EVOO increased the expression of miR-17 and miR-122 and reduced the circulating levels of miR-20a and miR-27b.

Conclusions: Different quantities of polyphenol-enriched EVOO modulated the postprandial circulating levels of cardiovascular relevant microRNAs, suggesting that some of the beneficial properties of olive oil-derived polyphenols could be mediated through the regulation of these.

P132-T | Modulation of plasma metabolites following a low-glycemic index diet

P. Hernández-Alonso*,†; S. Giardina*,†; D. Cañueto‡,§; J. Salas-Salvadó*,†; N. Cañellas‡,§; M. Bulló*,†

Human Nutrition Unit-URV-IISPV, Reus, Spain; †CIBEROBN-ISCIII, Madrid, Spain; ‡Metabolomics Platform-URV-IISPV, Tarragona, Spain; §CIBERDEM-ISCIII, Madrid, Spain

Background: Low glycemic index (LGI) diets consistently exert beneficial metabolic effects reducing glucose levels. However, their potential modulatory effect on other plasma metabolites has not been fully addressed in the medium term. We therefore examined whether a LGI diet improves a set of plasma metabolites related with different metabolic diseases.

Material and methods: We conducted a parallel randomized clinical trial with three intervention arms: a LGI, a high glycemic index (HGI) and a low fat (LF) diet. A total of 104 overweight or obese subjects were enrolled in the GLYNDIET study for 6 months. Blood samples were collected at baseline and at the end of the intervention. Plasma metabolomic profile, which mostly comprised lipid species and amino acids, was analyzed with three different approaches: GC/q-TOF (Q-TOF), LC/q-TOF and Nuclear Magnetic Resonance (NMR).

Results: Among the amino acids analyzed, serine levels were significantly increased following the LGI diet compared to both HGI and LF diets ($q = 0{\cdot}002$), whereas leucine and valine were reduced in the LGI vs LF diet ($q = 0{\cdot}015$ and $q = 0{\cdot}024$, respectively). A set of two sphingomyelins, two lysophosphatidylcholines and six phosphatidylcholines were significantly modulated in LGI diet compared to HGI or LF diet ($q < 0{\cdot}05$). We reported significant correlations between plasma amino acids and lipid species with parameters such as body weight, glucose, insulin and inflammatory markers. Even though multivariate analysis did not show a clear separation of the intervention periods, we reported a modulation of specific metabolites.

Conclusions: Our results suggest that a LGI diet may modulate a set of metabolites associated to derangements that take part in different metabolic diseases such as obesity, type 2 diabetes and hypertension. Further studies are needed to extrapolate our results to other populations.

P133-T | Ultra-processed food consumption and the incidence of depression in a mediterranean cohort: the seguimiento universidad de navarra project

C. Gómez-Donoso[*]; M.Á. Martínez-González[*,†,‡,§]; A. Romanos-Nanclares[*]; L. Ruiz-Estigarribia[*]; R. Mendonça[¶]; A. Sánchez-Villegas[†,**]; F. Lahortiga[††]; M. Bes-Rastrollo[*,†,‡]

[*]Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; [†]Biomedical Research Center Network on Physiopathology of Obesity and Nutrition (CIBERobn), Institute of Health Carlos III, Madrid, Spain; [‡]Navarra's Health Research Institute (IDISNA), Pamplona, Spain; [§]Department of Nutrition, Harvard TH Chan School of Public Health, Boston, USA; [¶]Department of Nutrition, School of Nursing, Federal University of Minas Gerais, Belo Horizonte, Brazil; [**]Nutrition Research Group, Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain; [††]Department of Psychiatry and Medical Psychology, University Clinic of Navarra, Pamplona, Spain

Background: Some available evidence suggests that high consumption of ultra-processed foods (UPFs) is associated with a higher risk of obesity, hypertension and metabolic syndrome. A growing body of research on the link between depression and other non-communicable diseases suggests that UPFs might also be associated with depression, which is among the leading causes of ill health and disability worldwide.

Material and methods: We prospectively evaluated the relationship between UPF consumption and the risk of depression in the SUN (Seguimiento Universidad de Navarra) project, a dynamic, prospective and multipurpose Spanish cohort. We included 14 907 Spanish university graduates initially free of depression, who were followed for a mean of 10·2 years. Consumption of UPFs (defined as food and drink products ready to eat, drink, or heat and made predominantly or entirely from processed items extracted or refined from whole foods or synthesized in the laboratory) was assessed at baseline through a validated semi-quantitative 136-item food-frequency questionnaire. Participants were classified as incident cases of depression if they reported a physician diagnosis or the use of antidepressant medication in at least one of the follow-up questionnaires.

Cox proportional hazards models were used to estimate adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for depression incidence.

Results: During follow-up, 608 incident cases of medically-diagnosed depression were identified. Participants in the highest quintile of UPF consumption had a higher risk of developing depression (adjusted HR [95% CI] = 1·55 [1·17–2·06]; P for trend = 0·002) than those in the lowest quintile after adjusting for potential confounders.

Conclusions: In a large prospective Spanish cohort, a significant positive association between UPF consumption and depression risk was observed among middle-aged adult university graduates. Findings were in line with expectations and previous results on dietary habits and risk of depression in the SUN project.

P134-T | Changes in gene expression of selected genes related with weight loss and cardiovascular risk after intervention with an energy-restricted Mediterranean diet and physical activity in the PREDIMED PLUS-Valencia study. Modulation by genetic variants

R.F. Carrión[*,†]; I. González-Monje[*,†]; C. Ortega-Azorín[*,†]; Eva.M. Asensio[*,†]; R. Barragán[*,†]; José.V. Sorlí[*,†]; O. Portolés[*,†]; D. Corella[*,†]

[*]Department of Preventive Medicine and Public Health. University of Valencia, Valencia, Spain; [†]CIBER Fisiopatología de la Obesidad y Nutrición. ISCIII, Madrid, Spain

Background: Mediterranean diet can produce favourable changes in the expression of genes related to cardiovascular risk. Our objective was to analyze if additional weight loss can increase the favourable changes in the expression of selected genes related to obesity, oxidative stress and diabetes. We also tested whether polymorphisms in the selected genes modulate gene expression.

Methods: We analyzed a subsample of participants (men and women aged 55–75 years) in the PREDIMED PLUS-Valencia randomized controlled trial. We selected 25 subjects in the intervention group (intensive intervention with energy restricted Mediterranean diet plus physical activity) and 25 subjects from the control group (Mediterranean diet advice). Weight was measured at baseline and after 6-months (M) intervention. RNA was isolated at baseline and 6M from blood. Gene expression was validated by individual RT-qPCR and fold change between control group and intervention group was calculated using the 2-ΔΔCt method. Selected genes were PER1 and CLOCK (circadian rhythm); CAT and GPX3 (oxidative stress); DDIT4 (DNA repair) and ARRDC3 (diabetes). We also analyzed polymorphisms in the selected genes genotyped by the Illumina Human OmniExpress Array.

Results: There were significant differences in weight loss at 6M between the intensive intervention and the control group (−5·06 and −0·62 kg, respectively; $P < 0·01$). We detected an increase in gene expression at 6M for the analyzed genes, but this increase was lower and not statistically significant in the control group separately. However, in the intervention group, we detected statistically significant changes in gene expression in a multivariate model for

the following genes: CLOCK, $P = 0{\cdot}008$; PER1, $P = 0{\cdot}034$; CAT, $P = 0{\cdot}006$; ARRDC3, $P = 0{\cdot}029$ and DDIT4, $P = 0{\cdot}033$. Borderline results were found for the CAT gene ($P = 0{\cdot}051$). Additional modulations were detected by some genetic variants (mainly by the rs1801260-CLOCK).

Conclusion: In addition to the Mediterranean diet, weight loss increased the favourable changes in gene-expression of the selected genes.

P135-T | Individual and combined effect of the adherence to the Mediterranean diet and physical activity on cardiovascular disease: the SUN cohort

I. Alvarez-Alvarez[*]; I. Zazpe[*,†]; Mr J.P. de Rojas[*]; A. Fernandez-Montero[*,‡]; M. Bes-Rastrollo[*,§]; M. Ruiz-Canela[*,§]; M. Hidalgo-Santamaría[*,¶]; M.Á. Martínez-González[*,§,**]

**Department of Preventive Medicine and Public Health, University of Navarra, IdiSNA, Pamplona, Spain; †Department of Nutrition and Food Sciences and Physiology, School of Pharmacy, University of Navarra, Pamplona, Spain; ‡Department of Occupational Medicine, University of Navarra Clinic, University of Navarra, Pamplona, Spain; §Biomedical Research Network Center for Pathophysiology of Obesity and Nutrition (CIBEROBN), Carlos III Health Institute, Madrid, Spain; ¶Department of Internal Medicine, University of Navarra Clinic, University of Navarra, Pamplona, Spain; **Department of Nutrition, Harvard TH Chan School of Public Health, Boston, United States of America*

Background: Benefits of adherence to the Mediterranean diet (MedDiet) and physical activity (PA) on cardiovascular disease (CVD) have been widely studied. We investigated the individual and combined effects of adherence to the MedDiet and PA on incident CVD in a population-based cohort.

Material and methods: We used data from 19 535 participants from a prospective cohort of Spanish university graduates, the SUN cohort, followed-up between December 1999 and May 2014. Adherence to the MedDiet was assessed using 4 different dietary scores, categorising them into tertiles of adherence. Engagement in PA was studied building an 8-item score, and measuring volume, intensity and frequency. Multivariable Cox regression models were used to study the individual and combined relationship of adherence to the MedDiet and PA with incident CVD (stroke, myocardial infarction or cardiovascular death).

Results: During a median follow-up of 10·4 years, we registered 140 CVD events. Compared to the lowest adherence to the MedDiet (≤3 in the Mediterranean Diet Score), greater adherence (6–9 points) was associated with lower CVD risk (multivariable adjusted hazard ratio [HR] = 0·36; 95% confidence interval [CI] 0·21–0·59). Engaging in higher levels of PA (6–8 points, measured with the 8-item score) showed a 22% non-significant decreased risk, while performing activities at moderate and vigorous intensity were strongly related with a statistically significant lower risk. When studying the joint effect of both exposures, higher adherence to the MedDiet and engaging in higher levels of PA showed an almost 80% reduction in risk (HR = 0·23; 95% CI 0·09–0·63). Likewise, participants who were greatly adhered to the MedDiet and performed exercise at a vigorous intensity showed a similar reduced risk (HR = 0·26; 95% CI 0·07–1·03).

Conclusions: The combined effect of adherence to the MedDiet and adopting an active lifestyle showed a synergistic effect on reducing incident CVD risk.

P136-T | Association of physical activity, sedentary behavior and Mediterranean diet with visceral adipose tissue accumulation in participants from the PREDIMED-PLUS trial: cross-sectional analysis

A.M.G. Panadés[*]; J. Konieczna[*]; I. Abete[†,‡]; M. Morey[*,†]; A. Colom[*,†]; M.A. Zulet[†,‡]; N. Babio[†,§]; Z. Vázquez[†,¶]; J. Salas-Salvadó[†,§]; J. Vidal[**,††]; R. Estruch[†,‡‡]; J. Alfredo Martínez[†,‡]; D. Romaguera[*,†]

**Instituto de Investigación Sanitaria Illes Balears (IdISBa), University Hospital Son Espases, Palma de Mallorca, Spain, Palma, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; ‡Department of Nutrition, Food Sciences and Physiology, Center for Nutrition Research, University of Navarra (UNAV), Pamplona, Spain; §Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Pere Virgili Institute for Health Research, Rovira i Virgili University, Reus, Spain; ¶Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; **CIBER Diabetes y enfermedades metabólicas (CIBERdem), Instituto de Salud Carlos III (ISCIII), Spain, Madrid, Spain; ††Department of Endocrinology, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain; ‡‡Department of Internal Medicine, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain*

Background/Objective: Visceral adipose tissue (VAT) is a strong predictor of cardiometabolic health, while lifestyle factors such as physical activity (PA) or adherence to the Mediterranean diet (MedDiet) may have a positive influence on VAT accumulation. The aim of this study was to assess the cross-sectional association between baseline levels of PA, sedentary behavior and adherence to the MedDiet with VAT accumulation in a sample of older individuals with overweight/obesity and metabolic syndrome.

Material and methods: The present investigation used baseline data of the PREDIMED-PLUS study including a sample of 1231 men and women aged 55–75 and 60–75 respectively. Levels of leisure-time PA (total, light, and

moderate-vigorous, in METs.min/day) and sedentary time (hour/d) were evaluated using validated questionnaires. Adherence to the MedDiet was evaluated using a 17-item energy-restricted (er) MedDiet screener. The chair test was used to estimate the muscle strength. VAT accumulation was assessed with DXA-CoreScan. Multivariable adjusted linear regression models were used to assess the association between our exposures and outcome.

Results: Total leisure-time PA (change in VAT per 100 METs.min/day: −24·3 g, 95% CI −36·7; −11·9), moderate-vigorous PA (−27·8 g, −4·8; −14·8), and chair test repeats (change in VAT per repeat −11·5 g, −20·1; −2·93) were associated with lower VAT accumulation (all *P*-values less than 0·001). Light PA, sedentary time and adherence to the 17-item erMedDiet were not significantly associated with VAT. However a significant interaction between PA and sedentary time was observed (*P* for interaction 0·012), with the greatest reduction in VAT (−251 g, −369; −135) observed in those with highest PA (above median) and lowest sedentary time (below median), compared to the opposite category.

Conclusions: In older subjects with overweigh/obesity and metabolic syndrome, PA (total and moderate-to-vigorous) and muscle strength were associated with VAT accumulation, mostly in those with fewer time spent on sedentary behaviours.

P137-T | Longitudinal analysis of changes in diet and changes in weight and waist circumference in an elderly population at high cardiovascular risk

V.P. Pérez[*,†]; D. Romaguera[*,†]; J. Konieczna[*]; C. Razquin[†,‡]; M. Morey[*,†]; M. Martín[*]; R. Estruch[†,‡]; E. Toledo[†,‡]; E.M. Asensio-Marquez[†,¶]; J. Salas-Salvadó[†,**]; M. Fito[†,††]; E. Gomez-Gracia**; E. Ros[†,§§]; M. Fiol[*,†]; J.M. Santos-Lozano[†,¶¶]; F. Aros[†,***]; L. Serra-Majem[†,†††]; X. Pinto[†,‡‡‡]; M.A. Martínez-González[†,‡]

**Instituto de investigación sanitaria Illes Balears (IdISBa), Palma, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; ‡Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; ¶Department of Preventive Medicine, University of Valencia, Valencia, Spain; **Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Pere Virgili Institute for Health Research, Rovira i Virgili University, Reus, Spain; ††Lipids and Cardiovascular Epidemiology Research Unit, Institut Municipal d'Investigació Mèdica (IMIM), Barcelona, Spain; §§Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain; ¶¶Department of Family Medicine, Primary Care Division of Sevilla, Centro de Salud San Pablo, Sevilla, Spain; ***Department of Cardiology, University Hospital of Alava, Vitoria, Spain; †††Institute for Biomedical Research, University of Las Palmas de Gran Canaria, Las Palmas, Spain; ‡‡‡Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain*

Background: Consumption of certain foods is associated with lower long-term weight gain and abdominal fat accumulation in healthy, young, non-obese participants. Whether the same foods prevent weight and waist gain in elderly, high-risk population is less known. We investigated how changes in dietary factors in the PREDIMED trial were associated with annual weight and waist circumference (WC) changes in elderly population.

Methods: A total of 7009 participants aged 55–70 years at high cardiovascular risk were included. Habitual diet was evaluated using a 137-items validated-FFQ, repeated yearly for five consecutive years. Foods, expressed as servings per day, were grouped into 31 food groups. Weight and WC were measured yearly (up to 5 years). The simultaneous association between yearly change in consumption of food groups and in weight and WC were evaluated using generalized estimated equations, adjusting for centre, age, sex, intervention arm, yearly measured energy intake and physical activity.

Results: Significant increments in weight were observed with change in consumption (in servings/d) of red meat (0·30 kg/y), alcoholic beverages (0·14), processed meat (0·14), other vegetable oils (0·10) and sweets (0·03); significant inverse associations with weight change were detected with change in the consumption of white meat (−0·20), low fat yogurt (−0·18), vegetables (−0·13), low fat milk (−0·08), fruit (−0·06) and extra-virgin olive oil (−0·03). Higher WC gain was observed with increment in consumption of snacks (0·31 cm/y), processed meats (0·28), artificially-sweetened (0·26) and sugar-sweetened (0·20) beverages, vegetables oils (0·19), alcoholic drinks (0·14), white bread (0·09), and sweets (0·04). Legumes (−1·32 cm/y), nuts (−0·35), vegetables (−0·33), natural juice (−0·26) and fruits (−0·11) significantly slowed down the gain in WC.

Conclusions: Ultra-processed foods, sodas, refined carbohydrates and red meats are associated with higher weight and WC gain, whereas plant foods and some dairy products are associated with less gain in weight and WC.

P138-T | Body adiposity indicators and cardiometabolic risk: cross-sectional analysis in participants from the PREDIMED-PLUS trial

J. Konieczna[*]; I. Abete[†,‡]; A.M. Galmés[*]; N. Babio[†,§]; M.A. Zulet[†,‡]; E. Toledo[†,¶]; A. Colom[*,†]; P. Buil-Cosiales[†,**]; M. Fiol[*,†]; J. Vidal[††,‡‡]; J. Vera[§§]; R. Estruch[†,¶¶]; J. Salas-Salvadó[†,§]; J. Alfredo Martínez[†,‡]; D. Romaguera[*,†]

**Instituto de Investigación Sanitaria Illes Balears (IdISBa), University Hospital Son Espases, Palma de Mallorca, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Madrid, Spain; ‡Department of Nutrition, Food Sciences and Physiology, Center for Nutrition Research, University of Navarra (UNAV), Pamplona, Spain; §Human Nutrition Unit, University Hospital of Sant Joan de Reus, Department of Biochemistry and Biotechnology, Pere Virgili Institute for Health Research, Rovira i Virgili University, Reus, Spain; ¶Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; **Osasunbidea, Servicio Navarro de Salud, Pamplona, Spain; ††CIBER Diabetes y enfermedades metabólicas (CIBERdem), Instituto de Salud Carlos III (ISCIII), Madrid, Spain; ‡‡Department of Endocrinology, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain; §§Institut Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; ¶¶Department of Internal Medicine, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain*

Background: Excess adiposity is associated with poor cardiometabolic (CM) health. To date, several techniques and adiposity indicators have been developed to determine adiposity. This study compared the ability of traditional anthropometric, as well as standard and novel DXA-derived parameters related to overall and regional adiposity, to evaluate CM risk.

Material and methods: Using the cross-sectional design in the context of the PREDIMED-PLUS trial, 1207 Caucasian older men and women with overweight/obesity and metabolic syndrome (MetS) were assessed. At baseline, anthropometry- and DXA-measured parameters of central, visceral, peripheral and central-to-peripheral adiposity together with comprehensive set of CM risk factors were obtained. Partial correlations and areas under the ROC curve (AUC) were estimated to compare each adiposity measure with CM risk, separately for men, women and in the overall sample.

Results: DXA-derived indicators, other than percentage of total body fat, showed stronger correlation (rho 0·101–0·206, $P < 0·001$) with CM risk than anthropometric indicators, after controlling for age, diabetes and medication use. In both sexes, DXA-derived visceral adipose tissue measures (VAT, VAT/Total fat, visceral-to-subcutaneous fat) together with lipodystrophy indicators (Trunk/Legs fat and Android/Gynoid fat) were strongly and positively correlated ($P < 0·001$) with glycated hemoglobin (HbA1c), the triglyceride and glucose index (TyG), triglycerides (TG), the ratio TG/HDL-cholesterol (TG/HDL-C), and were inversely related to HDL-C levels ($P < 0·001$). Furthermore, in AUC analyses for both sexes, VAT/Total fat showed the highest predictive ability for abnormal HbA1c levels (AUC = 0·629), VAT for TyG (AUC = 0·626), both lipodystrophy indicators for TG (AUCs = 0·556), Trunk/Legs fat for HDL-C (AUC = 0·556) and TG/HDL-C (AUC = 0·581).

Conclusions: DXA regional adiposity measures offer advantages beyond traditional anthropometric and DXA overall adiposity indicators for CM risk assessment in older overweight/obese subjects with MetS. In particular, in both sexes, visceral adiposity better stratifies individuals at risk for insulin resistance and diabetes, and indicators of lipodystrophy better predict markers of dyslipidemia.

P139-T | Study of systematic-level variations caused by swimming as a model of physical exercise

L. Fernández-Calleja; A.D. Luis; S. Rodríguez-González; B. de Luxán-Delgado; A. Rubio-González; I. Menéndez-Valle; B. Caballero; I. Vega-Naredo; A. Coto-Montes

Department of Morphology and Cell Biology, University of Oviedo, Oviedo, Spain

High intensity physical exercise of elite athletes can induce physical and physiological benefits or produce negative impact when exercise is exacerbated, since this induce excessive increase in the organism of the so-called "reactive oxygen species" (ROS). ROS increase activates the body's antioxidant defense lines, including two essential enzymes for this system, superoxide dismutase (SOD) and catalase (CAT). Both are responsible for neutralizing the oxidative potential of ROS, as long as they act together and balanced.

In order to analyze the systemic effect of training, in this study we have used subjects that play an elite sport (swimming) during their pre-competition training phase compared with sedentary subjects, as control.

ROS cause cellular damage by oxidation. Their interaction with the different cellular components causes molecular inactivation and, therefore, the functional activity stop. These phenomena of incapacitation of molecules could have unwanted effects after years of competition that can be abolished with adequate oxidant defense.

Our results suggest that, due to a mismatch in SOD and CAT activity, the high levels of ROS generated in swimmers have not been properly neutralized. The non-neutralized radicals have caused damage both erythrocyte and plasmatic levels, which directly carries a detrimental effect on the oxygen transport to cells. This increase in ROS during training, while it is important for improving their personal best, can induce deleterious effects in the future.

Acknowledgments: This work was supported by PI13/02741, PI17/02009 and GRUPIN14-071.

P140-T | Plasma branched-chain/aromatic amino acids and risk of type-2 diabetes after a Mediterranean diet intervention: a case-cohort study within the PREDIMED trial

M. Ruiz-Canela*,†,‡; M. Guasch-Ferré§; E. Toledo*,†,‡; C.B. Clish¶; C. Razquin*,†,‡; L. Liang**; Dong.D. Wang§; D. Corella‡,††; R. Estruch‡,‡‡; Á. Hernáez‡,§§; E. Yu§; E. Gómez-Gracia‡,¶¶; Y. Zheng§; F. Arós‡,***; D. Romaguera‡,†††; C. Dennis¶; E. Ros‡,‡‡; J. Lapetra‡,‡‡‡; L. Serra-Majem‡,§§§; C. Papandreu‡,¶¶¶; O. Portoles‡,††; M. Fitó‡,§§; J. Salas-Salvadó‡,¶¶¶; F.B. Hu§,****,††††; Miguel.A. Martínez-González*,†,‡,§

**University Of Navarra, Pamplona, Spain; †IdiSNA, Navarra Institute for Health Research, Spain; ‡CIBER Fisiopatología de la Obesidad y Nutrición (CIBER Obn), Instituto de Salud Carlos III, Madrid, Spain; §Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, USA; ¶Broad Institute of MIT and Harvard University, Cambridge, USA; **Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, USA; ††Department of Preventive Medicine, University of Valencia, Valencia, Spain; ‡‡Hospital Clinic, University of Barcelona, Spain; §§Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, Barcelona, Spain; ¶¶Department of Preventive Medicine, University of Malaga, Málaga, Spain; ***Department of Cardiology, University Hospital of Alava, Vitoria, Spain; †††IdISBa, Balearic Islands Health Research Institute, Palma de Mallorca, Spain; ‡‡‡Department of Family Medicine, Primary Care Division of Sevilla, San Pablo Health Center, Sevilla, Spain; §§§Research Institute of Biomedical and Health Sciences and Medical School University of Las Palmas de Gran Canarias, Las Palmas de Gran Canarias, Spain; ¶¶¶Human Nutrition Department, IISPV, Universitat Rovira I Virgili, Reus, Spain; ****Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA; ††††Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, USA*

Background: Branched-chain (BCAAs) and aromatic amino-acids (AAs) are associated with type 2 diabetes. However, interactions of BCAA/AA with dietary interventions have not been evaluated. We assessed the associations between baseline and 1-year changes of BCAA/AA and incident type-2 diabetes in the "PREvención con DIeta MEDiterránea" (PREDIMED) study, a trial examining health effects of Mediterranean diet (MedDiet).

Material and methods: We included 251 incident cases of diabetes and a random sample of 694 participants (641 non-cases and 53 overlapping cases) in a case-cohort study nested within the PREDIMED trial. Participants were allocated to a MedDiet+extra-virgin olive oil (n = 273), a MedDiet+nuts (n = 324) or a control diet (n = 295). We used LC-MS/MS to measure plasma levels of amino acids.

Results: Elevated plasma levels of individual BCAA/AA were associated with higher diabetes risk after a median follow-up of 3·8 years: the multivariable hazard ratios (HRs) for the highest vs lowest quartile ranged from 1·32 for phenylalanine (95% confidence interval (CI): 0·90–1·92, *P*-trend = 0·015) to 3·29 for leucine (95% CI: 2·03–5·34, *P*-trend<0·001). One-year increases in a BCAA score were associated with higher diabetes risk in the control group, with HR per SD = 1·61 (95% CI: 1·02–2·54), but not in the MedDiet groups (*P*-interaction <0·001). The MedDiet+extra-virgin olive oil was associated with reduced BCAAs levels after 1-yr of intervention (*P* = 0·005).

Conclusions: Higher baseline BCAAs and their 1-year increases were associated with higher diabetes risk. A MedDiet rich in extra-virgin olive oil significantly reduced BCAA levels and attenuated the positive association between plasma BCAA and incident diabetes.

P141-T | Public open spaces and weather as modulators factors on physical activity objectively measured in older adults

A.C. Fernández*,†; M. Fiol*,†; J. Wärnberg‡; J.C.B. Marín‡; M. Compa§; M. Ruiz¶; M.D. Sanmartín**; M. Morey†; M.Á. Martín†; A. Galmés†; V. Pereira*,†; D. Romaguera*,†

**CIBER Fisiopatología de la Obesidad y Nutrición (CIBER-OBN), Palma de Mallorca, Spain; †Institut de Investigació Sanitària Illes Balears (IDISBA), Palma de Mallorca, Spain; ‡School of Health Sciences. University of Málaga-IBIMA, Málaga, Spain; §Instituto Español de Oceanografía (IEO), Palma de Mallorca, Spain; ¶Universitat de les Illes Balears (UIB), Palma de Mallorca, Spain; **Servicio de Salud de las Islas Baleares (Ib-Salut), Palma de Mallorca, Spain*

Background: Access to public open spaces (POS) and weather conditions are important determinants on the practice of physical activity and sedentary behaviors, however, little is known about their effect among elderly populations in the Mediterranean basin. Here we assess the cross-sectional association of access to POS and accelerometer measured physical activity and sedentary behaviors, and the influence of weather conditions in older adults participants from the PREDIMED-PUS-Baleares.

Method: There were a total of 220 participants in the PREDIMEDPLUS-Baleares living within the city limits of Palma de Mallorca who wore an accelerometer. Exposure to POS was determined as distance to the closest park from the participants' home location. Physical activity and sedentary behaviors were measured as daily accumulative practice minutes using an accelerometer. Exposure to weather conditions was determined of each near station as the mean daily cumulative precipitation (mm) and mean temperature (°C) during the time period each participant was wearing the accelerometer.

Results: Evaluations were made on 218 participants (51·4% women, aged 65·19 ± 4·7 years). The average distance to the closest park was 281·6 (±396·3) meters and weather condition was 1·0 (±2·2) (mm) for precipitation and 20·0 (±5·4) (°C) for temperature. The average physical

activity (LMVPA) and sedentarism was respectively 335·4 (±62·2) and 424·9 (±81·6) daily accumulative minutes. Preliminary results indicate no significant association between distance to closest park and Physical activity or sedentary behaviors. However, significant association between weather conditions and Physical activity or sedentary behaviors was observed in this population.

Conclusion: When promoting physical activity practice, it is important to consider the plausible environmental determinants that may affect this practice. In this elderly population living in a Mediterranean city, distance to the closest park did not influence PA practice, but weather conditions did appear to be an important factor related to active ageing.

P142-T | Evaluation of bias in reviews about olive oil

M.M. García[*]; M.G. Conesa[†]; I.P. Yanez[‡]; M.A.M. Gonzalez[§]

[*]Servicio Navarro De Salud-Osasunbidea, Pamplona, Spain; [†]Hospital Universitario de Alcorcón, Alcorcón, Spain; [‡]Hospital Severo Ochoa, Leganés, Spain; [§]Universidad de Navarra, Pamplona, Spain

Introduction: Olive oil is the main source of fat and health-promoting component of the Mediterranean diet. The search for consistency in the association between olive oil and the reduction of diseases has led to many published reviews on the properties of olive oil for the prevention of several conditions. Amidst these studies, there seems to be an excessive focus on the positive properties of olive oil, without attention to counter-arguments, caution in the hypotheses or limitations of the available evidence. We assessed whether recent review articles on olive oil and prevention of disease have a bias towards reporting beneficial results, and to what extent the reported results were focused on olive oil as the key component of the Mediterranean food pattern, in comparison to assessments of the overall dietary pattern.

Methods: We searched PubMed for reviews about human studies published between 2013 and 2017 containing 'olive oil' in their title.

Results: We found 40 reviews; 26 had, as objectives, to summarize the beneficial properties of olive oil. Among them, 30 reviews were conducted in Mediterranean countries. The most common topics were the anti-inflammatory properties of olive oil (7) and metabolic syndrome (6). Only five articles were systematic reviews including meta-analysis. Only seven mentioned any limitation in their results or a need for further investigation. On average, 37% (±22%) of the references in the reviews mentioned olive oil, while only 15% (±18%) alluded to the Mediterranean dietary pattern.

Conclusions: Most reviews seemed to acknowledge as definite evidence the beneficial properties of olive oil. Reviews seemed more focused on olive oil as an isolated nutrient vs as a part of the Mediterranean dietary pattern. Although olive oil is a significant part of this dietary pattern, perhaps more focus should be directed to the overall pattern instead of highlighting this single component.

P143-T | Self-perceived level of competitiveness, tension and dependency and lifestyles in the sun cohort

M.Á.M. González; I. Zazpe; A. Sánchez-Villegas; P. Molero; S. Santiago; F. Lahortiga; C.R. Unzueta

Universidad De Navarra, Pamplona, Spain

Background: The relationship between personality factors and specific lifestyles is an area of interest. In this context, we aimed to assess the differences on lifestyles in participants of Seguimiento Universidad de Navarra (SUN) cohort, according to their level of self-perceived competitiveness, tension and dependency.

Material and methods: Food consumption, nutrient intake, eating attitudes, physical activity, sedentariness, and alcohol and tobacco consumption were assessed among 15 346 Spanish adults through a questionnaire administered at baseline. Personality traits items were collected also at baseline and included three questions (competitiveness, tension and dependency). For each question there were 11 possible answers ranging from 0 (more conformist, relaxed or autonomous) to 10 (more competitive, tense or dependent). Each personality trait was categorized into three groups: low level (1–4), moderate level (5–6) and high level (7–10). ANOVA and Chi square tests were used to assess the association between personality traits and lifestyles, and Benjamini-Hochberg's q-value procedure for controlling false discoveries.

Results: Participants with high level of self-perceived competitiveness consumed more vegetables and fish but less refined grains, they had higher protein intake and healthier eating attitudes. They were more physically active and less likely to be smokers. Participants with high level of tension or dependency were less physically active, and subjects more dependent had also poorer adherence to the Mediterranean diet.

Conclusions: In this cohort, self-perceived personality traits showed associations with dietary patterns, nutrient intake, eating attitudes, physical activity, sedentary

lifestyles and smoking status. Particularly, high level of competitiveness appears to be related with healthier lifestyles. The use of short questions about self-perceived level of competitiveness, psychological tension and dependency can contribute to obtain additional interesting information when assessing health risk behaviours in adults.

P144-T | A healthy lifestyle score and the risk of depression

L. Ruiz-Estigarribia[*]; M.Á. Martínez-González[*,†,‡]; J. Díaz-Gutiérrez[*]; C. Gómez-Donoso[*]; A. Romanos-Nanclares[*]; A. Sánchez-Villegas[§]; F. Lahortiga-Ramos[¶]; M. Bes-Rastrollo[*,†]

[*]Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra, Pamplona, Spain; [†]Biomedical Research Network Center for Pathophysiology of Obesity and Nutrition (CIBEROBN), Carlos III Health Institute, Madrid, Spain; [‡]Department of Nutrition, Harvard TH Chan School of Public Health, Boston, USA; [§]Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain; [¶]Department of Psychiatry and Medical Psychology, University Clinic of Navarra, Pamplona, Spain

Background: Depression is considered to be a multifactorial disease. There is evidence that a range of lifestyle factors are involved in the pathogenesis of depression and many of these factors can be modified for the potential prevention of depression. The aim of this study was to assess the association between a healthy-lifestyle score and the incidence of depression in the SUN (Seguimiento Universidad de Navarra) cohort study.

Methods: The SUN project is a dynamic prospective cohort of Spanish university graduates. We followed 15 093 participants initially free of any history of depression. Based on a score that was previously associated with lower risk of clinical cardiovascular events and based on the existing scientific evidence; we calculated a healthy-lifestyle score from 0 to 7 points, by giving one point to each of the following components: never smoking, physical activity (>20 METs-h/wk), Mediterranean diet adherence (≥4/8 points), low body mass index (≤22 kg/m^2), moderate alcohol intake (women 0·1–5 g/day, men 0·1–10 g/day), no binge drinking (never >5 drinks/day), and working (≥40 hour/wk).

Results: During a median of 10·4 years, we observed 794 incident cases of medically-diagnosed depression. The median age of participants was 36·7 years ±11·7 and 60% were women. The highest category (5–7 factors) showed a significant 21% relative reduction in the risk of developing depression compared to the lowest category (0–2 factors) (multivariable-adjusted hazard ratio = 0·79; 95% CI: 0·65–0·98) (*P* for trend = 0·021).

Conclusions: In this cohort of Spanish university graduates, adopting a healthy-lifestyle was associated with a lower risk of depression. This index including seven simple healthy lifestyle habits may be useful for a more integrative approach to depression prevention.

P145-T | Sugar-sweetened beverage consumption and incidence of breast cancer: the SUN project

A. Romanos-Nanclares[*]; A. Gea[*,†,‡]; I. Gardeazábal[*,§]; R. Sánchez-Bayona[*,§]; L. Ruiz-Estigarribia[*]; C. Gomez-Donoso[*]; M.A. Martinez-González[*,†,‡,¶]; E. Toledo[*,†,‡]

[*]Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; [†]Biomedical Research Center Network on Physiopathology of Obesity and Nutrition (CIBERobn), Institute of Health Carlos III, Madrid, Spain; [‡]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain; [§]Department of Oncology, University of Navarra Clinic, Pamplona, Spain; [¶]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, USA

Background: Breast cancer (BC) incidence is increasing worldwide. The risk of BC duplicates each decade until the menopause and immediately upon it starts to decelerate. Although causes of BC are not fully understood, it has been suggested that higher insulin resistance may lead to an increased risk of BC. Among the dietary factors that can influence insulin resistance, sugar-sweetened beverages (SSB) have been related to a higher insulin resistance and type 2 diabetes. However, the association between SSB and BC has not been widely studied. Thus, we evaluated the association between baseline consumption of SSB and the incidence of BC among relatively young women in a cohort of Spanish university graduates.

Methods: We evaluated 10 709 middle-aged, Spanish female university graduates from the Seguimiento Universidad de Navarra (SUN) cohort, initially free of BC. SSB consumption was collected at baseline using a 136-item semi-quantitative food-frequency questionnaire. Incidence of BC was self-reported and then confirmed by a trained oncologist using medical records. We also consulted the National Death Index to identify deaths due to BC. We fitted Cox regression models to assess the relationship between categories of SSB consumption and the incidence of BC during follow-up. Stratified analyses by menopausal status were conducted.

Results: During a total of 107 126 person-years of follow-up, 83 incident cases of BC were confirmed. Among postmenopausal women, regular consumption of SSB was significantly associated with a greater risk of developing BC (Hazard ratio: 2·60; 95% confidence interval: 1·08, 6·27) in the fully adjusted model, compared to women who never or seldom consumed SSB. No association was found for

premenopausal women (Hazard ratio: 0·90; 95% confidence interval: 0·49, 1·68).

Conclusions: Even though the number of cases was small, we observed an association between SSB consumption and higher postmenopausal breast cancer risk. Nonetheless further longitudinal larger studies are needed to support this association.

P146-T | Plasma trimethylamine-N-oxide and related metabolites are associated to type 2 diabetes risk in the PREDIMED trial

C. Papandreou[*]; M. Bulló[*]; Y. Zheng[†]; M. Ruiz-Canela[‡]; E. Yu[†]; M. Guasch-Ferré[†]; E. Toledo[‡]; C. Clish[§]; D. Corella[¶]; R. Estruch**; E. Ros[††]; M. Fitó[‡‡]; F. Arós[§§]; M. Fiol[¶¶]; J. Lapetra***; L. Serra-Majem[†††]; E. Gómez-Gracia[‡‡‡]; L. Liang[§§§]; G. Fragkiadakis[¶¶¶]; C. Razquin[‡]; F. Hu[†]; J. Salas-Salvadó[*]

**Human Nutrition Unit, Faculty of Medicine and Health Sciences, Institut d'Investigació Sanitària Pere Virgili, Rovira i Virgili University, Reus, Spain, Reus, Spain; †Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA, Boston, USA; ‡University of Navarra, Department of Preventive Medicine and Public Health, IdisNA, Pamplona, Spain; §Broad Institute of MIT and Harvard University, Cambridge, USA; ¶Department of Preventive Medicine, University of Valencia, Valencia, Spain; **Department of Internal Medicine, Department of Endocrinology and Nutrition Institut d'Investigacions Biomediques August Pi Sunyer (IDI-BAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain; ††Lipid Clinic, Department of Endocrinology and Nutrition Institut d'Investigacions Biomediques August Pi Sunyer (IDI-BAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain; ‡‡Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, Barcelona, Spain; §§Department of Cardiology, University Hospital of Alava, Vitoria, Spain; ¶¶Institute of Health Sciences IUNICS, University of Balearic Islands and Hospital Son Espases, Palma de Mallorca, Spain; ***Department of Family Medicine, Primary Care Division of Sevilla, San Pablo Health Center, Sevilla, Spain; †††Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain; ‡‡‡Department of Preventive Medicine, University of Málaga, Málaga, Spain; §§§Departments of Epidemiology and Statistics, Harvard T.H. Chan School of Public Health, Boston, USA; ¶¶¶Department of Nutrition and Dietetics, Technological Education Institute of Crete, Crete, Greece*

Background: The role of trimethylamine-N-oxide (TMAO) in type 2 diabetes (T2D) is currently partially understood and controversial. We aimed to investigate associations between TMAO and related metabolites with type 2 diabetes (T2D) risk in subjects at high risk of cardiovascular disease.

Material and methods: This is a case-cohort design study within the PREDIMED study, with 251 incident T2D cases and a random sample of 694 participants (641 non-cases and 53 overlapping cases) without T2D at baseline (median follow-up: 3·8 years). We used liquid chromatography-tandem mass spectrometry to measure plasma TMAO, L-carnitine, betaine, lyso-phosphatidylcholine (LPC) and lyso-phosphatidylethanolamine (LPE) species, phosphocholine, alpha-glycerophosphocholine, choline, at baseline and 1-year. We examined associations using weighted Cox proportional hazard models; accounting for the weighted case-cohort design by the Barlow method.

Results: After adjusting for recognized T2D risk factors and multiple testing, individuals in the highest quartile of baseline TMAO and alpha-glycerophosphocholine had lower risk of T2D; hazard ratio (HR) 0·52 (95% CI 0·29, 0·89), and 0·46 (95% CI 0·24, 0·89), respectively. The HR (95% CI) comparing the extreme quartiles of betaine was 0·41 (0·23, 0·74). Similar trends were observed for C16:0 LPC, C18:1 LPC, C18:0 LPC, C20:4 LPC, C22:6 LPC, C18:1 LPC plasmalogen and C16:0 LPE. After correcting for multiple comparisons, participants in the highest quartile of 1-year changes in C18:1 LPC plasmalogen levels had lower T2D risk as compared to the reference quartile.

Conclusions: Whether the associations between plasma TMAO and certain metabolites levels with T2D risk reflect its pathophysiology or represent an epiphenomenon need to be elucidated.

P147-T | Assessing dietary sustainability at the community level

C. Ruano-Rodriguez[*]; P. Momo-Cabrera[*]; A. Ortiz-Andrelluchi[*]; E. Gónzalez-Padilla[*]; A. Alvarez-Falcón*,[†]; L. Serra-Majem*,[‡]

**Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria (ULPGC), Las Palmas De Gran Canaria, Spain; †University Hospital of Gran Canaria "Doctor Negrín", Las Palmas de Gran Canaria, Spain; ‡UNESCO Chair of Local Health Systems and Food Systems, Spain*

Background: The Mediterranean Diet is considered to be the epitome of what a Sustainable Diet means. "The Island on Your Plate" is a communication project that intends to draw attention towards the gastronomic diversity of the island of Gran Canaria (Canary Islands, Spain) and to encourage a more sustainable diet by adapting the local habits to a Mediterranean-like Diet. A survey has been developed with the aim to investigate consumer's dietary habits and food shopping preferences in Gran Canaria.

Material and methods: The survey will be piloted in twenty subjects at two SPAR stores located in both rural and urban areas of the island. Validity and Reliability of the piloted survey will be tested through SPSS analyses. A final model will be developed and distributed around the island through SPAR's weekly catalogue expecting to recollect at least data from 3000 participants.

Results: Results of the survey are expected to provide representative data of diverse socioeconomic levels and food

shopping behaviours in Gran Canaria. Moreover, we had elaborated the Decalogue of a Healthy Diet in the Community: 10 keys for a healthy life and world. This Decalogue was edited in a video format with the aim to be a useful tool to disseminate healthy eating habits, and its relation with science and our food heritage. Finally, an online platform was created to divulge information regarding other initiatives—both public and private—that promote the ideology behind food sustainability.

Conclusions: This project has manifested the possibility to make a significant change on Gran Canaria's dietary pattern to promote and encourage a sustainable diet of proximity that is balanced, varied, and healthy and to bring it closer to the Mediterranean Diet pattern. Results gathered from the survey will enable to evaluate sustainability in food shopping and dietary practices in Gran Canaria.

P148-T | Effects of a walnut-enriched diet for 2 years on oxylipins derived from arachidonic and alpha-linolenic acids

M. Cofan[*,†,‡]; A. Sala-Vila[*,†,‡]; A. Checa[§]; C.E. Wheelock[§]; T.-M.F. Simoes[†,‡]; M. Serra-Mir[†,‡]; S. Rajaram[¶]; J. Sabaté[¶]; E. Ros[*,†,‡]

**Ciber Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain; †Lipid Clinic, Department of Endocrinology and Nutrition, Hospital Clínic de Barcelona, Barcelona, Spain; ‡Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Barcelona, Spain; §Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden; ¶Department of Nutrition, School of Public Health, Loma Linda University, Loma Linda, US*

Background: Oxylipins are biologically active oxidation products of dietary polyunsaturated fatty acids (PUFAs) that play a role in cardiovascular disease and aging. Research on dietary strategies to modulate serum oxylipins is incipient. Results from clinical trials indicate that fish oil-derived n-3PUFAs promote the formation of anti-inflammatory and vasodilatory oxylipins, but there are little clinical data on oxylipins derived from alpha-linolenic acid (ALA), the vegetable n-3PUFA. Walnuts are a sustainable source of ALA. We investigated whether a diet enriched with walnuts at 15% energy (30–60 g/d) for 2 y would shift circulating oxylipins to a more vasculoprotective pattern in healthy elders (aged 63 to 79 y) compared to a control diet.

Materials and methods: The Walnuts and Healthy Aging study is a two-center (Barcelona and Loma Linda, California) parallel trial designed to test the effects of walnuts on age-related diseases. In a sub-study of the Barcelona site participants, randomly assigned to the walnut diet (n = 64) or the control diet (n = 51), we used HPLC-MS to measure serum concentration of 53 oxylipins at baseline and 2 year. We also determined the red blood cell (RBC) proportion of ALA by gas-chromatography as a measure of compliance.

Results: After 2 years, RBC ALA increased significantly ($P < 0{\cdot}001$) in the walnut group compared with the control group. Consumption of walnuts decreased arachidonic acid-derived products of soluble epoxide hydrolase, namely 5·6-, 8·9-, 11·12- and 14·15-dihydroxy-eicosatrienoic acid ($P = 0{\cdot}029$, $0{\cdot}013$, $0{\cdot}019$ and $0{\cdot}076$ vs control, respectively). Compared to control diet, the walnut diet also resulted in significant increases of ALA-derived oxylipins 9-, 13-hydroxy-octadecatrienoic acid, 9-oxo-octadecatrienoic acid, and 12,13-epoxy-octadecatrienoic acid ($P < 0{\cdot}001$, all).

Conclusions: Long-term walnut consumption was associated with decreased levels of arachidonic acid-derived oxylipins with vasoconstrictor and pro-inflammatory properties and increased ALA-derived oxylipins with putative vasculoprotective effects. These results add novel mechanistic evidence to the well-known cardioprotective effects of walnuts.

P149-T | Comparative study of the oxidative status of subcutaneous and omental fat and their contribution to adipose tissue dysfunction and insulin resistance

M.D.C.N. Ruiz[*]; R.G. Ruiz[*,†]; A. Díaz-Ruiz[‡]; J.R. Peinado[§]; A. Membrives[¶]; J.L. Miranda[†,**]; Md.M. Malagón[*,†]

**Department of Cell Biology, Physiology, and Immunology/University of Córdoba (UCO)/Maimónides Biomedical Research Institute of Córdoba (IMIBIC)/Reina Sofia University Hospital (HURS), Córdoba, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Córdoba, Spain; ‡Translational Gerontology Branch, National Institute on Aging (NIH), Baltimore, USA; §Department of Medical Sciences/University of Castilla-La Mancha (UCLM), Ciudad Real, Spain; ¶Department of Surgery/Maimónides Biomedical Research Institute Of Córdoba (IMIBIC)/Reina Sofia University Hospital (HURS), Córdoba, Spain; **Lipids and Atherosclerosis Unit/University of Córdoba (UCO)/Maimónides Biomedical Research Institute of Córdoba (IMIBIC)/Reina Sofia University Hospital (HURS), Córdoba, Spain*

Background: Obesity is a multifactorial disease characterized by adipose tissue (AT) dysfunction, which is commonly associated to insulin resistance (IR). At the molecular level, adipose tissue dysregulation is related, among other processes, to obesity-triggered oxidative stress. In this scenario, carbonylation is one of the most common oxidative modifications of proteins, yet poorly characterized, which serves as a marker of oxidative stress. Herein, we aimed at profiling carbonylated proteins in

omental and subcutaneous AT from morbidly obese patients with different degrees of insulin sensitivity (normoglycemia–NG-, insulin resistance–IR-, or type 2 diabetes–T2D) in order to identify potential biomarkers of oxidative damage in obesity and metabolic disease.

Materials and methods: Lipid peroxidation and carbonylated protein profiles in omental and subcutaneous AT were identified and quantified by proteomic analyses (1D and 2D-PAGE). Intracellular ROS were measured using 2,7′-dichlorofloorescein diacetate, and oxidative status was measured as superoxide dismutase 1 (SOD1) and glutathione synthase (GS) content by immunoblotting.

Results: Lipid peroxidation was higher in IR and T2D vs NG in omental and subcutaneous fat. 2D-PAGE revealed both common (serotransferrin, vimentin, actin, annexin A2) and distinct (subcutaneous AT: carbonic anhydrase, α-crystallin B; omental AT: α-1-antitrypsin, tubulin) carbonylated proteins in the two fat depots. Increased intracellular ROS levels in omental fat were related to high levels of antioxidant enzymes in IR, whereas these changes were not detected in paired samples of subcutaneous fat.

Conclusions: Although lipid peroxidation content is similar in both depots, our studies show differences in both carbonylated proteins and antioxidant markers between subcutaneous and omental fat, and suggest that oxidative damage in omental fat is greater than in subcutaneous fat in IR. Among the targets of protein oxidative modification, serotransferrin may represent a mediator contributing to adipocyte dysfunction in obesity.

Funding: MINECO/FEDER (BFU2015-70454-REDT; BUF2016-76711-R); FPU14/04994; JJAA/FEDER (PI-0200/2013; PI-0159-2016); FIS (PIE14_00005), CIBERobn (ISCIII).

P150-T | Mediterranean diet and health outcomes in the SUN cohort

M.Á.M. Gonzalez; S. Carlos; C. de la Fuente; M. Bes-Rastrollo; C. Razquin; A. Rico-Campà; M. Ruiz-Canela

Universidad De Navarra, Pamplona, Spain

Background: The Mediterranean Dietary Pattern (MedDiet) has been linked to many beneficial health effects. We have reviewed the main findings of the Seguimiento Universidad de Navarra (SUN) cohort study regarding MedDiet and the risk of major chronic diseases.

Material and methods: The SUN project is an open cohort in which 22 782 Spanish university graduates have participated since 1999. Data on diet, lifestyle and clinical diagnosis are collected at baseline and every two years. Diet is assessed using a 136-item semi-quantitative food frequency questionnaire, previously validated in Spain. The collection of biological samples began in 2008. Saliva samples are requested from a subpopulation of participants aged 55 years and over.

Results: After reviewing 23 studies from the SUN study focused on the MedDiet effects on chronic disease, a high MedDiet adherence is associated with a reduced incidence of overall all-cause mortality, fatal and non-fatal cardiovascular disease, diabetes, weight gain, metabolic syndrome, depression, cognitive decline and nephrolithiasis. An inverse dose-response relationship was found for many of these associations. The MedDiet was also associated with lower average heart rate, a mitigation in the harmful effect of overweight/obesity on CVD, and the attenuation of the effects of obesity on diabetes. A suggestion that the MedDiet may enhance fertility was found. None of the scores evaluated showed a significant inverse association with gestational diabetes or hypertension, although reduced changes in systolic and diastolic blood pressure were observed.

Conclusions: We conclude that this cohort has provided evidence that a high MedDiet adherence is associated with a reduced incidence of overall all-cause mortality, fatal and non-fatal cardiovascular disease, diabetes, weight gain, metabolic syndrome, depression, cognitive decline and nephrolithiasis

P151-T | Lipidome patterns and the risk of type 2 diabetes in the PREDIMED study

C. Razquin*,†,‡; E. Toledo*,†,‡; C. Clish§; M. Ruiz-canela*,†,‡; C. Dennis§; D. Corella‡,¶; E. Ros‡,**; R. Estruch‡,††; M. Guasch-Ferre‡,‡‡; E. Gomez-Gracia‡,§§; M. Fito‡,¶¶; E. Yu‡‡; J. Lapetra‡,***; D. Wang‡‡; D. Romaguera‡,†††; L. Liang‡‡‡,§§§; F. Aros‡,¶¶¶; C. Papandreou****; A. Diek§; M. Bullo‡,****; L. Serra-Majem‡,†††††; S.-S. Jordi‡,****; F.B. Hu‡‡,‡‡‡‡; M.A. Martinez-Gonzalez*,†,‡,‡‡

**Department of Preventive Medicine and Public Health, University Of Navarra, Pamplona, Spain; †IdiSNA, Navarra Institute for Health Research, Pamplona, España; ‡CIBER Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III, Madrid, Spain; §Broad Institute of MIT and Harvard University, Cambridge, USA; ¶Department of Preventive Medicine, University of Valencia, Valencia, Spain; **Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomediques August Pi Sunyer (IDI-BAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain; ††Department of Internal Medicine, Institut d'Investigacions Biomediques August Pi Sunyer (IDI-BAPS), Barcelona, Spain; ‡‡Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Spain; §§Department of Preventive Medicine, University of Malaga, Malaga, Spain; ¶¶Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, Barcelona, Spain; ***Department of Family Medicine, Distrito Sanitario Atención Primaria Sevilla, Centro de Salud Universitario San Pablo, Sevilla, Spain; †††Instituto de Investigación Sanitaria de Palma (IdISPa), University Hospital of Son Espases, Palma de Mallorca, Spain; ‡‡‡Department of Epidemiology Harvard T.H. Chan School of Public Health, Boston, USA; §§§Department of Biostatistics Harvard T.H. Chan School of Public Health, Boston, USA; ¶¶¶Department of Cardiology, University Hospital of Alava, Vitoria, Spain; ****Human Nutrition Unit, Faculty of Medicine and Health Sciences, Institut d'Investigació Sanitària Pere Virgili, Rovira i Virgili University, Reus, Spain; ††††Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, University of Las Palmas de Gran Canaria, Las Palmas, Spain; ‡‡‡‡Channing Division for Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA*

Background: Type 2 diabetes (T2D) entails a worldwide epidemic. Obesity and dyslipidemia are included among T2D main known risk factors. However, the specific lipid molecular changes that may lead to insulin resistance and T2D remain unknown. Our aim was to identify lipidome patterns longitudinally associated with T2D, including analyses of their 1-year changes and the subsequent T2D risk in the context of a Mediterranean diet intervention trial.

Materials and methods: In this case-cohort study, 889 participants were included, 639 were in the subcohort (including 53 overlapping incident cases) and 197 were incident T2D cases (total incident cases = 53 + 197). Participants were followed-up during 3·8 years (median). We repeatedly measured 302 plasma known lipid metabolites at baseline and after 1-year of intervention. Principal component analysis was used to identify lipidome factors. Six factors were significantly associated with T2D. Considering common patterns among factors, lipid molecular species were grouped (summed) into scores depending on lipid classes.

Results: We found that baseline lysophosphatidylcholines and lysophosphatidylethanolamines (grouped as LP), phosphatidylcholine-plasmalogens (PC-PL), sphingomyelins (SM) and cholesterol esters (CE) were inversely associated with the risk of T2D (*P* for linear trend = <0·001, <0·001, <0·001 and <0·001 respectively; adjusted for sex, age and intervention group). On the contrary, baseline triacylglycerols (TAG), diacylglycerols (DAG) and phosphatidylethanolamines (PE) were associated with a higher risk of T2D (*P* for linear trend = 0·009, <0·001, <0·001, respectively). Although the results were not statistically significant, the associations between one year changes and the subsequent risk of T2D also pointed in the same directions.

Conclusions: Two plasma lipid patterns, comprising different lipid classes, were associated with the risk of T2D: one pattern including LP, PC-PL, SM, CE and CE-A was linked to a reduced risk of T2D while another pattern composed of TAG, TAG-A, DAG and PE was associated with a higher T2D risk.

P152-T | Adherence to Mediterranean diet reduces the prevalence of obesity and abdominal obesity at 4 years and the risk of incident abdominal obesity at 9 years in children of the INMA study

L. Notario-Barandiaran*,†; M.G. de la Hera*,†; E. Pereda‡,§,¶; A. Fernández-Somoano†,**; M. Guxens†,††,‡‡,§§; S. González-Palacios*,†; D. Valera-Gran†; L. Torres-Collado†; L.S. Marina‡,§,¶; A. Tardón†,**; M. Vrijheid†,††,‡‡; C. Iñiguez*,¶¶; J. Vioque*,†; E.M.N. Muñoz*,†

**CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain; †Department of Public Health, History of Medicine and Gynecology, Universidad Miguel Hernández, Alicante Institute for Health and Biomedical Research (ISABIAL - FISABIO Foundation), Alicante, Spain; ‡Instituto de Investigación Sanitaria, BIODONOSTIA, San Sebastian, Spain; §Departamento de Medicina Preventiva y Salud Pública, Universidad del País Vasco UPV-EHU, Spain; ¶Public Health Division of Gipuzkoa, BioDonostia Research Institute, San Sebastian, Spain; **Department of Medicine, Universidad de Oviedo, Oviedo, Asturias; ††ISGlobal, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain; ‡‡Universitat Pompeu Fabra, Barcelona, Spain; §§Department of Child and Adolescent Psychiatry/Psychology, Erasmus University Medical Centre–Sophia Children's Hospital, Rotterdam, The Netherlands; ¶¶Epidemiology and Environmental Health Joint Research Unit (FISABIO-Universitat Jaume I-Universitat de València), Valencia, Spain*

Introduction: The prevalence of obesity in childhood has increased worldwide in the last few decades. It has been suggested that adherence to a Mediterranean dietary (MD) pattern may reduce the risk obesity, particularly among adults but the evidence for children is scarce. We assess the association between MD and the prevalence of obesity

and abdominal obesity in children 4 years old, and the incidence of obesity and abdominal obesity until the age of 9 years.

Methods: We analyzed data of children of the prospective cohort INMA study, from the age of 4 years (n = 1826) until the age of 9 years (n = 1539). We assessed diet using a validated food frequency questionnaire and the adherence to MD through the relative Mediterranean Diet score (rMED) although excluding alcohol intake. Weight, height and waist circumference (WC) were measured. Children were classified as overweight and obese according to their body mass index (BMI) Cole et al, 2000, and their 90% percentile of WC. We used logistic regression and Cox proportional hazard models to estimate effect measures and confidence intervals.

Results: The prevalence of obesity and abdominal obesity were 5% and 9% respectively. The incidence of obesity and abdominal obesity from the age of 4 to 9 years were 6% and 9% respectively. The average score of rMED adherence was 8 points. In multiple analysis, a 2 point increase in rMED adherence was associated with a lower risk of obesity OR = 0·77 (IC 95%: 0·64; 0·92) and abdominal obesity OR = 0·86 (IC 95%: 0·72; 1·02). A 2-point increase in rMED adherence was associated with a lower risk of developing abdominal obesity, RR = 0·82 (IC 95%: 0·68; 0·99), but not with obesity incidence (P = 0·724).

Conclusion: The adherence to a MD at the age of 4 years is associated with a lower prevalence of obesity and abdominal obesity, and a lower risk of developing abdominal obesity later until the age of 9 years.

P153-T | Validity of glycated hemoglobin in diagnostic of dysglycemia in patients with impaired fasting glucose respect to the oral glucose tolerance test

J.M. Santos-Lozano*,†; Md.C. Jiménez‡; A. Angel-Lugo‡; A.M. Gómez*; A. Vilches-Arenas§; I. Rivera-Benítez*; F.J. García-Corte*,†; M. Ortega-Calvo*,†; C. Toro-Cortés*; J. Lapetra*,†

**Department of Family Medicine, Distrito Sanitario Atención Primaria Sevilla, Servicio Andaluz de Salud, Sevilla, Spain; †CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; ‡Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI), Sevilla, Spain; §Department of Preventive Medicine and Public Health, Universidad de Sevilla, Sevilla, Spain*

Background: Patients with impaired fasting glucose (IFG) have an increased risk of diabetes in the next few years. This risk greatly increases if also presents an impaired glucose tolerance (IGT). Therefore, it is advisable to rule out IGT, even diabetes, from its standard test: 2-hour, 75 g oral glucose tolerance test (OGTT), in these patients. OGTT has several disadvantages: low reproducibility, fasting status, and time availability, while glycated hemoglobin (A1C) has the advantage that it is a standardized test and does not require fasting. Our objective is to determine the validity of A1C as a diagnostic test of dysglycemia (IGT and diabetes) in patients with IFG, according to ADA criteria.

Material and methods: Cross-sectional study, nested in a randomized controlled trial (Clinical Trial PREDIABOLE: ISRCTN03372660). 197 patients with IFG. Outcome variables: OGTT, fasting glucose, A1C. Statistical analysis: Kappa-Index, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The best predictive cut-off value for A1C for detecting patients with IGT and new diabetes was identified using the optimal sensitivity and specificity values determined by the receiver operating characteristics (ROC) curve.

Results: 197 patients: 50% male. Average age: 60,59,0. Average body mass index: 31,64,4. Kappa Index: 0·130. Sensitivity: 93% (95% CI: 87–99). Specificity: 22% (95% CI: 13–30). PPV: 48·5% (95% CI: 41–56). NPV: 80% (95% CI: 64–95). ROC curve: 0·676 (95% CI: 0·601–0·752; P = 0·0001). Optimal cut-off point of A1C: 5·95 (sensitivity 76%, specificity 51%).

Conclusions: (i) The cut-off established by ADA for A1C of 5·7% does not discriminate patients with dysglycemia, featuring high sensitivity but low specificity, low PPV and high NPV. (ii) A1C value (combining highest values of sensitivity and specificity) is 5·95, more consistent with proposed by International Expert Committee (6,0). (iii) Poor value of ROC curve of A1C to diagnose disglucemics states. (iv) Very low concordance between OGTT and A1C.

P154-T | Effects of resveratrol and pterostilbene on NOV/CCN3 in adipose tissue and liver from rats fed an obesogenic diet

S. Gómez-Zorita*,†; J. Trepiana*; s.N. Romo*; A. Fernández-Quintela*,†; M.P. Portillo*,†

**Nutrition and Obesity Group, Department of Nutrition and Food Science, University of the Basque Country (UPV/EHU), VITORIA-GASTEIZ, Spain; †CIBERobn Physiopathology of Obesity and Nutrition, Institute of Health Carlos III, VITORIA-GASTEIZ, Spain*

Introduction: NOV/CCN3 is a matricellular protein expressed in various tissues (adipose tissue, liver, adrenal cortex, kidney and muscle). Recently, it has been reported that this adipokine is involved in the development of some clinical alterations (obesity, tumorigenesis or fibrosis). In

this context, we aimed to determine the effect of resveratrol and its methoxy analogue, pterostilbene, on NOV/CCN3 in adipose tissue and liver from rats fed an obesogenic diet.

Methods: 36 male growing Wistar rats were divided into four experimental groups (n = 9): standard diet (CC) or fed an obesogenic diet, supplemented with resveratrol (RSV) (30 mg/kg body weight/d), pterostilbene (PT) (30 mg/kg body weight/d) or without phenolic compound supplementation (HFS). After 6 weeks of treatment, rats were sacrificed and liver and adipose tissue from different locations were dissected. Triglyceride content was measured by Folch method. In liver, subcutaneous and epididymal adipose tissues NOV/CCN3 gene and protein expressions were analysed by RT-PCR and western blot analysis respectively.

Results: The obesogenic diet increased perirenal, epididymal, mesenteric and subcutaneous adipose tissue weights. Both resveratrol and pterostilbene-treated rats showed reduced internal adipose tissue weights, but not subcutaneous adipose depot.

As far as liver is concerned, rats fed the HFS diet showed increased liver triglycerides. This phenomenon was partially prevented by resveratrol, but not by pterostilbene. In HFS-fed rats, NOV/CCN3 gene expression decreased in epididymal fat depot (−52%) compared with the CC group. In this tissue, PT-treated rats showed lower NOV/CCN3 protein expression (−53%) and RSV-treated animals higher gene expression (+381%). In subcutaneous depot and in liver, no differences were observed in NOV/CCN3 among groups.

Conclusion: Despite their similarity in chemical structure, resveratrol and pterostilbene act differently on NOV/CC3 in adipose tissue. In view of these results, further studies are needed to determine the potential involvement of NOV/CC3 on the anti-obesity effects of these molecules.

P155-T | Effects of resveratrol, a phenolic compound found in foods typical in the Mediterranean diet, on brown adipose tissue

I. Milton-Laskibar*,†; L. Aguirre*,†; U. Etxeberria‡; F.I. Milagro†,‡; J.A. Martínez†,‡; M.P. Portillo*,‡

**Nutrition and Obesity Group, Department of Nutrition and Food Science, University of the Basque Country (UPV/EHU), VITORIA-GASTEIZ, Spain; †CIBERobn Physiopathology of Obesity and Nutrition, Institute of Health Carlos III, Madrid, Spain; ‡Department of Nutrition, Food Sciences and Physiology, Centre for Nutrition Research, University of Navarra, Pamplona, Spain*

Introduction: Brown adipose tissue (BAT) is the main thermogenic tissue in the body. Its activity is mediated by uncoupling protein 1 (UCP1), which dissipates the energy as heat. Some stimuli can induce thermogenesis, among them cold, diet and some bioactive compounds, such as resveratrol. This polyphenol has been reported to act as an energy restriction mimetic molecule.

Objective: To analyze the effects of resveratrol on BAT mitochondrial activity and thermogenesis, and to compare these effects with those induced by energy restriction.

Methods: Six-week-old rats were fed a high-fat high-sucrose diet for 6 weeks, and then divided into four groups and fed a standard diet for 6 additional weeks: control group (C), resveratrol group (RSV, resveratrol 30 mg/kg/d), restricted group (R, 15% energy restriction) and combined group (RR, 15% energy restriction and resveratrol 30 mg/kg/d). The activities of carnitine palmitoyltransferase 1a (CPT1a) and citrate synthase (CS), protein expression of Sirtuin 3 (SIRT3), mitochondrial transcription factor A (TFAM), UCP1 and nuclear respiratory factor 1 (NRF1), as well as the acetylation of PGC1α were analyzed.

Results: Increased CPT1a activities were found in the three treated groups, but only the RR group showed greater CS activity. R and RR groups showed higher SIRT3 protein expression. TFAM and NRF1 protein expressions increased in RSV, R and RR groups. In the case of UCP1, only the RSV group showed increased protein expression. Finally, decreased acetylation of PGC1α was found in RSV and RR groups.

Conclusions: Resveratrol administration and energy restriction induce the expression of signaling proteins involved in mitochondriogenesis in BAT. However, the ability of resveratrol to increase UCP1 protein expression is greater than that observed in energy restriction. Thus, the mechanisms of action of these two strategies are not the same. When the two interventions are combined no greater effects are observed.

P156-T | Dietary folate intake and cardiometabolic risk in participants of the PREDIMED-PLUS randomized trial

E.M.N. Muñoz[*,†]; M.G. de la Hera[*,†]; S. Gonzalez-Palacios[*,†]; L. Torres-Collado[*,†]; M. Fitó[‡,§]; D. Corella[‡,¶]; J.A. Martinez[‡,**]; D. Romaguera[‡,††]; J. Wärnberg[‡,‡‡]; J. López-Miranda[‡,§§]; R. Estruch[‡,¶¶]; A. Bueno-Cavanillas[*,***]; F. Aros[‡,†††]; J.A. Tur[‡,‡‡‡]; F. Tinahones[‡,§§§]; L. Serra-Majem[‡,¶¶¶]; V. Martin[*,****]; J. Lapetra[‡,††††]; C. Vázquez[‡,‡‡‡‡]; X. Pintó[‡,§§§§]; J. Vidal[¶¶¶¶,*****]; L. Daimiel[†††††]; M. Delgado-Rodríguez[*,‡‡‡‡‡]; P. Matía[§§§§§]; E. Ros[‡,¶¶¶¶¶]; J. Salas-Salvado[‡,******]; M.A. Martinez-Gonzalez[‡,††††††,‡‡‡‡‡‡]; J. Vioque[*,†]

*CIBER Epidemiología y Salud Pública (CIBEResp), Instituto de Salud Carlos III (ISCIII), Madrid, Spain; †Miguel Hernandez University, ISABIAL-FISABIO, Alicante, Spain; ‡IBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Spain; §Lipids and Cardiovascular Epidemiology Research Unit, Institut Municipal d'Investigació Mèdica (IMIM), Barcelon, Spain; ¶Department of Preventive Medicine, University of Valencia, Valencia, Spain; **Department of Nutrition and Food Sciences, Physiology and Toxicology, University of Navarra, Pamplona, Spain; ††Institute of Health Sciences (IUNICS), University of Balearic Islands, Palma de Mallorca, Spain; ‡‡School of Nursing, University of Málaga, Málaga, Spain; §§Department of Internal Medicine, University of Córdoba-IMIBIC, Cordoba, Spain; ¶¶Department of Internal Medicine, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain; ***Department of Preventive Medicine, University of Granada, Granada, Spain; †††Department of Cardiology, University Hospital of Alava, Vitoria, Spain; ‡‡‡Department of Fundamental Biology and Health Sciences, University of Balearic Islands, Palma de Mallorca, Spain; §§§Department of Endocrinology, University Hospital, University of Málaga, Málaga, Spain; ¶¶¶Institute for Biomedical Research, University of Las Palmas de Gran Canaria, Las Palmas, Spain; ****Division of Preventive Medicine, University of León, León, Spain; ††††Department of Family Medicine, Primary Care Division of Sevilla, Centro de Salud San Pablo, Sevilla, Spain; ‡‡‡‡Department of Endocrinology, Fundación Jiménez-Díaz, Madrid, Spain; §§§§Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain; ¶¶¶¶CIBER Diabetes y enfermedades metabólicas (CIBERdem), Instituto de Salud Carlos III (ISCIII), Spain; *****Department of Endocrinology, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain; †††††IMDEA, Madrid, Spain; ‡‡‡‡‡Division of Preventive Medicine, University of Jaén, Jaén, Spain; §§§§§Department of Endocrinology, Clinic Hospital of Madrid, Madrid, Spain; ¶¶¶¶¶Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Barcelona, Spain; ******Human Nutrition Unit, IISPV, Universitat Rovira i Virgili, Reus, Spain; ††††††Department of Preventive Medicine and Public Health, University of Navarra-IDISNA, Pamplona, Spain; ‡‡‡‡‡‡Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, USA

Background: Folate and folic acid (FA) are two forms of vitamin B9 that may have different metabolic pathways to reduce homocysteine in blood and the risk of cardiovascular disease (CVD). Although FA use has been associated with decreased risk of CVD, the evidence about the effects of folate on cardiovascular risk is scarce. In this study, we explore the association between dietary folate intake and cardiometabolic risk among subjects with overweight and metabolic syndrome of the PREDIMED-PLUS trial at baseline.

Methods: We analyzed data from 6598 participants with overweight/obesity and with at least three components of metabolic syndrome in the PREDIMED-PLUS trial at baseline. Dietary folate intake (per 100 mcg/day and in quintiles) was estimated using a validated food frequency questionnaire. We calculated a cardiometabolic risk score (CRS) using the standardized values as shown in the formula: [(body mass index + waist-to-height ratio)/2] + [(systolic blood pressure + diastolic blood pressure)/2] + plasma fasting glucose − HDL cholesterol + plasma triglycerides. The CRS as a continuous variable was the outcome variable. We explored the association between CRS and folate intake using multiple robust regressions models with Huber method.

Results: We observed inverse associations between dietary folate intake (per 100 mcg/day) and CRS (β = −0·14; CI 95%: −0·21 to −0·08), waist-to-height ratio (β = −0·04; CI 95%: −0·06 to −0·02), systolic blood pressure (β = −0·03; CI 95%: −0·06 to −0·01) and plasma fasting glucose (β = −0·04; CI 95%: −0·06 to −0·02). After adjusting for the adherence to a Mediterranean dietary score, the associations did not change substantially.

Conclusion: This study suggests that higher folate intake is associated with a lower cardiometabolic risk score probably through several components such as waist to height ratio, systolic blood pressure and plasma fasting glucose in high-risk subjects of the PREDIMED-PLUS trial.

Financial support: FIS FEDER (PI14/01206; 17/01441; ERC 2013-2018 340918)

P157-T | Effect of a mediterranean diet on the primary prevention of atrial fibrillation and major cardiovascular events in hypertensive patients with high cardiovascular risk: results of ICFAMED randomized trial

J. Lapetra[*,†]; J.M. Lozano-Rodríguez*; L. Miró-Moriano[†]; M. Ortega-Calvo[*,†]; J.M. Santos-Lozano[*,†]; F.J. García-Corte[*,†]; M.R. Caballero-Valderrama[‡]; C. Domínguez-Espinaco[§]; S. Vaquero-Diaz[†]; A. Santos-Calonge*

*Department of Family Medicine, Distrito Sanitario Atención Primaria Sevilla, Servicio Andaluz de Salud, Sevilla, Spain; †CIBER de Fisiopatoogía de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; ‡Department of Cardiology, Hospital Universitario Virgen del Rocío, Servicio Andaluz de Salud, Sevilla, Spain; §Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI), Sevilla, Spain

Background: Our objective is to evaluate the effect of a MedDiet, compared with a low fat diet (LFD), on the incidence of this group of diseases in hypertensive patients with high cardiovascular risk (CVR) in a situation of primary prevention (PP).

Material and methods: Clinical trial (ISRCTN27497769), of dietary intervention, randomized, controlled, simple blind, and multicenter, performed entirely in primary care. 180 participants (62·8% women), hypertensive patients with a high CVR in PP. Random assignment to 2 intervention groups (IG): 90 to MedDiet; 90 to LFD. For at least 2 years, they received dietary advice (individual and group) every three months. Primary outcome variable: variable composed of AF, stroke, IC and HF. The occurrence of POV was detected by the annual ECG, periodic contact with patients and their family physicians, and consultation medical history. Intention-to-treat analysis. Descriptive, comparative analysis, calculation of hazard ratios (HR) and survival analysis. Level of significance was $P < 0{\cdot}05$.

Results: After a mean follow-up of 27·6 ± 5 months there were 16 events: IG-MedDiet: 5 (FA: 2; IC: 2; stroke: 1); IG-LFD: 11 (FA: 6; CI: 2; stroke: 3). The crude rate for the occurrence of events per 1000 patient-months of follow-up was 1·97 (95% CI: 0·6–4·6) for the IG-MedDiet and 4·51 (95% CI: 3–8.1) for IG-LFD. The HR for patients with IG-MedDiet compared to IG-LFD was 0·44 (95% CI: 0·15–1·26, $P > 0{\cdot}05$). Survival analysis showed the protective effect of MedDiet vs LFD after 15 months of follow-up.

Conclusions: In hypertensive patients at high CVR, in a PP situation, the 2·3-year follow-up of a MedDiet compared to a LFD resulted in a reduction in the risk of cardiovascular complications related to hypertension (AF, stroke, IC and HF) of 56%. This effect was mainly due to the decrease in the incidence of AF and stroke.

P158-T | Ultraprocessed food consumption and all-cause mortality: the University of Navarra Follow-Up (SUN) cohort

A. Rico-Campà*,†,‡; I. Alvarez-Alvarez*; R. de Deus Mendonça*,§; C. de la Fuente-Arrillaga*,†,‡; M.A. Martínez-González*,†,‡,¶; M. Bes-Rastrollo*,†,‡

Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; †CIBERobn Physiopathology of Obesity and Nutrition, Institute of Health Carlos III (ISCIII), Madrid, Spain; ‡IdiSNA, Navarra Institute for Health Research, Pamplona, Spain; §Department of Nutrition, Federal University of Minas Gerais, Brasil; ¶Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, USA

Background: Ultraprocessed food (UPF) consumption has increased in the past decade. Because of its nutritional composition evidence suggests a potential positive association between UPF consumption and the risk of all-cause mortality. Few prospective studies have investigated this relationship.

Material and methods: We evaluated the association between UPF consumption and the risk of mortality in a prospective Spanish cohort of university graduates, the SUN study.

We used data from 19 887 participants followed-up between December 1999 and May 2014 for a median of 10·4 years with a retention rate of 90·9%.

UPF consumption (defined as food and drink products ready to eat, drink, or heat and made predominantly or entirely from processed items extracted or refined from whole foods or synthesized in the laboratory) was assessed with the use of a validated semiquantitative 136-item food-frequency questionnaire. We adjusted UPF consumption for energy intake using the residuals method. Participants were classified according to their energy-adjusted UPF consumption into quartiles. Cox proportional hazards models were used to estimate adjusted Hazards Ratio (HR) and 95% confidence intervals (CI) for all-cause mortality.

Results: We registered 351 deaths. Those participants in the highest quartile of UPF consumption had an 80% relatively higher risk of all-cause death compared to those in the first quartile (multivariable adjusted HR = 1·80; 95% CI: 1·29–2·51) with a significant dose-response trend (P-trend = 0·001). For each additional serving of UPF consumption the risk of mortality relatively increased 7% (adjusted HR = 1·07; 95% CI: 1·02–1·12).

Conclusions: Ultraprocessed food consumption was associated with a significant increased risk of all-cause mortality in a prospective cohort of Spanish middle-aged adult university graduates. Further longitudinal studies are needed to confirm our results.

P159-T | Comparison of anti-inflammatory properties of molecular complex of pectin with acetylsalicylic acid and same of acetylsalicylic acid

A. Vyshtakalyuk; O. Lenina; L. Mironova; S. Minzanova

A.E. Arbuzov Institute of Organic and Physical Chemistry - Subdivision of the Federal State Budgetary Institution of Science "Kazan Scientific Center of Russian Academy of Sciences", Kazan, Russian Federation

Now the searching of new effective and non-toxic anti-inflammatory preparations as well as reduce of toxicity of known anti-inflammatory preparation are very relevant. The molecular complexes of pectin and carboxylic acids [1] including complexes with acetylsalicylic acid [2] were synthesized in our previous studies. It was shown that complex formation of carboxylic acids and pectin leads to pronounced reduce of toxicity of carboxylic acids. Moreover, signs of irritation and ulcerogenic action of acetylsalicylic acid on the stomach were minimal when the complex of

pectin and acetylsalicylic acid was used [2]. In present work we investigated anti-inflammatory properties of molecular complex of pectin with acetylsalicylic acid in comparison with equivalent dose of acetylsalicylic acid.

Experiment was carried out on males of outbred rats, 6 animals in each group. Carrageenan was injected under the plantar aponeurosis of the hind limb. In 30 minutes the tested preparations were administered orally in the doses 100 and 200 mg/kg of acetylsalicylic acid and 1000 and 2000 mg/kg of pectin complex correspondingly. The severity of edema was estimated with using plethysmometer Ugo Basile, the pain—by the mechanical analgesiometer.

It was shown that the anti-inflammatory action of complex is more pronounced if the dose 1000 mg/kg was used. Anti-edema effect of pectin complex was the same as effect of acetylsalicylic acid in the equivalent dose 100 mg/kg. Analgesic properties of complex were more pronounced: the pain level was less in 2 times in the group administered with pectin complex in comparison with equivalent dose of acetylsalicylic acid.

References:

1. Minzanova S.T., Mironov V.F., Vyshtakalyuk A.B. et al. Doklady Chemistry. 2010. 434(1):249–252.

2. Minzanova S.T., Mironov V.F., Vyshtakalyuk A.B. et al. 2013. 452(1):230–233.

The reported study was funded by RFBR according to the research project no 18-013-01177.

P160-T | Use of LC-HRMS based metabolomics to elucidate beer polyphenol health effects in a human cross over intervention trail

P.Q. Rada[*,†]; G.C. Blanch[‡,§]; O. Jáuregui[¶]; R. Estruch[§]; R.L. Raventos[*,†]

[*]Department of Nutrition and Food Science-XARTA-INSA, School of Pharmacy, Campus de l'Alimentació Torribera, University of Barcelona, Barcelona, Spain; [†]CIBER Physiopathology of obesity and nutrition (CIBEROBN), Institute of Health Carlos III, Spain; [‡]Cardiovascular Research Center (CSIC-ICCC), Barcelona, Spain; [§]Department of Internal Medicine, Hospital Clinic, Institute of Biomedical Investigation August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain; [¶]Scientific and Technological Centers of the University of Barcelona (CCiTUB), Spain

Introduction: Moderate alcohol consumption has been inversely associated with incidence of cardiovascular risk factors and all-cause mortality, independently of the type of alcoholic beverage consumed. However, the results of some studies indicate that fermented alcoholic beverages, like wine or beer, may provide additional protective effects due to their polyphenolic content. The aim of this work was to study the urinary metabolomics changes of participants that consumed beer, non-alcoholic beer and gin in order to evaluate metabolic shifts related to the beverage type and discovery of new potential biomarkers of beer consumption.

Methods: A crossover trial with 33 men at high cardiovascular risk were randomized to receive beer (30 g alcohol/d), the equivalent amount of polyphenols in the form of non-alcoholic beer, or gin for 4 weeks. Diet and physical exercise were carefully monitored. Urine samples were analyzed by LC-HRMS (LTQ-Orbitrap). A combination of univariate statistical analysis, multivariate analysis (PLS-DA), data-dependent MS/MS scan and accurate mass database matching was used to measure the effect of beer, non-alcoholic beer and gin intake in the urinary metabolome. Moreover, the metabolomics data results were correlated with other cardiovascular risk factors such as plasmatic nitric oxide, expression of several adhesion molecules and representative biomarkers of inflammation.

Results and conclusions: 10 metabolites were identified discriminating between interventions and baseline point. After the beer and non-alcoholic beer interventions, we observed increased urine excretion of hop α-acids like humulone, cohumulone and oxyhumilinic acid, as well as 2,3-dihydroxy-3-methylvaleric acid, and 1,2,3,4-tetrahydro-1-methyl-β-carboline-3-carboxylic acid. After the gin and beer interventions, metabolites from the alcohol detoxification process like ethyl sulfate, 2-phenylethanol glucuronide, and ethyl glucuronide increased in urine. Moreover, beer and non-alcoholic beer intake increase the excretion of two endogenous metabolites: hydroxyadipic acid linked to fatty acid oxidation and 4-guanidinobutanoic acid which was also correlated with a decrease in urinary creatinine.

P161-T | Correlation between circulating microRNAs and chronic kidney disease in patients with and without type 2 diabetes

A. Muendlein[*,†]; K. Geiger[*]; A. Leiherer[*,†,‡]; C.H. Saely[*,†,§,¶]; J. Ebner[*]; E.-M. Brandtner[*]; D. Zanolin[*]; A. Vonbank[*,†,§]; A. Mader[*,†,§]; B. Larcher[*,†,§]; P. Fraunberger[†,‡]; H. Drexel[*,†,¶,**]

*[*]Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; [†]Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; [‡]Medical Central Laboratories, Feldkirch, Austria; [§]Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; [¶]Division of Angiology, Swiss Cardiovascular Center, Berne, Switzerland; **Drexel University College of Medicine, Philadelphia, United States*

Background: MicroRNAs (miRNAs) are small non-coding RNAs that usually function as intracellular repressors of target genes involved in many pathophysiologic processes. MiRNAs can also be detected outside cells including circulating cell-free body fluids. Circulating miRNAs have been proposed repeatedly in the literature as new attractive biomarkers in many diseases, including type 2 diabetes (T2DM) and chronic kidney disease (CKD). T2DM is

closely linked to the development of CKD and it is unclear if putative associations between miRNAs and CKD differ in patients with or without T2DM.

Material and methods: We therefore investigated the association between a panel of 40 candidate-miRNAs and CKD (based on estimated glomerular filtration rate) in 120 angiographied coronary patients with (n = 65) and without (n = 55) T2DM, respectively. P-values of less than 0·05 were regarded as statistically significant after correction for multiple testing.

Results: In the total patient cohort, miR-320a and miR-320b were significantly increased and miR-451a, miR-106b-5p, miR-25-3p, miR-20a-5p, miR-19b-3p, miR-16-5p, and miR-140-3p were significantly decreased in patients with CKD (corrected P-values ranging between 0·003 and 0·045). In the subpopulation of patients without T2DM associations between miR-451a, miR-106b-5p, miR-25-3p, miR-20a-5p, miR-19b-3p, miR-16-5p, and miR-140-3p and CKD were still significant (corrected P-values ranging between 0·011 and 0·039). In addition the association between miR-19a-3p and miR-99b-5p with CKD was significant in non-diabetic patients (corrected P-values = 0·039 and 0·021, respectively). None of the investigated miRNAs were significantly associated with CKD in patients with T2DM, at least after correction for multiple testing.

Conclusion: We conclude that numerous circulating miRNAs are significantly associated with CKD and that this association may be masked by the prevalence of T2DM.

PRECISION NUTRITION

P091-F | Effect of alcoholic and non-alcoholic beer in the microRNA profile of plasma and macrophages in high cardiovascular risk patients

V. Micó*; L. Daimiel*; L. Berninches*; V. Loria-Kohen†; I. Espinosa-Salinas†; J.M. Ordovás*,‡

Nutritional Genomics and Epigenomics Group, IMDEA Food, CEI UAM + CSIC, 28049 Madrid, Spain; †Platform for Clinical Trials in Nutrition and Health. GENYAL, IMDEA Food, CEI UAM + CSIC, 28049 Madrid, Spain; ‡Nutrition and Genomics Laboratory, JM-USDA Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts, MA, -02111, USA., Boston, United States

Background: Beer is one of the most popular beverages in Europe. Moderate beer intake is associated with cardiovascular health factors such as increased HDL plasma concentration, decreased LDL oxidation and anti-inflammatory properties. Beer is rich in polyphenols and it could be included in the diet of healthy people or people with cardiovascular disease or obesity. Despite these health associations, the mechanisms related to these beneficial effects of beer consumption have not been studied in depth. We aimed to describe if beer consumption modifies circulating and macrophage levels of microRNAs involved in cardiovascular health.

Material and methods: We recruited seven men from 25 to 65 years old, non-smokers, with at least 2 or more criteria of Metabolic Syndrome. The intervention was performed with two washout periods of 7 days each and two intervention periods of beer consumption (alcoholic and non-alcoholic beer) for 14 days each. We analyzed the expression of a selected panel of 62 microRNA in plasma and macrophage samples of these patients.

Results: We obtained several microRNAs significantly differentially expressed between interventions and basal periods. We observed miR17-92 cluster upregulated consistently in both beer types. In plasma, miR29 and miR423-5p were upregulated with alcoholic beer consumption and let-7e and miR10 with non-alcoholic beer consumption while miR 320 was downregulated. In macrophages, MiR423-5p, miR21 and miR3 were upregulated with alcoholic beer consumption while miR15, miR130 or miR133 were upregulated after the non-alcoholic beer consumption.

Conclusion: We obtained significant expression changes in several miRNAs with beer consumption. These miRNAs are related to endothelial function, inflammation, or atherosclerosis and their modification after beer consumption suggest that the reported cardio-protective effect of beer intake could be mediated by the regulation of these microRNAs. Further studies are needed to demonstrate the mechanisms by which beer modulates these microRNAs experimentally.

P092-F | Complex nutritional management in a patient with two rare diseases: methymalonacidemia (MMA) and maple syrup disease (MSUD)

N. Karabul; V. Boettcher; A. Nassour; A. Mann

Endokrinologikum Frankfurt, Medical Center, Frankfurt, Germany

The female patient was diagnosed with MMA and MSUD at age 3 by early onset seizures. Since then she was on low protein diet and Vitamin B12 for her MMA, additionally carnitine, biotin, thiamine for her MSUD, since branched chain amino acids have to restricted. The child was thereafter admitted on multiple occasions with acute decompensation (eg hyperammonemia) and managed as per protocol.

MMA presents with lethargy, acidosis, hypoglycemia/hyperglycemia, ketosis, and recurrent episodes. Severe MMA has poor prognosis in most cases with limited life expectancy. Early recognition and appropriate treatment of acute

crises are necessary. Metabolic stroke can sometimes occur in the absence of acute metabolic decompensation, so meticulous neurological examination at each visit is useful. MSUD is an inborn error of metabolism caused by defects in the branched-chain α-ketoacid dehydrogenase complex, which results in elevations of the branched-chain amino acids (BCAAs) in plasma, α-ketoacids in urine, and production of the pathognomonic disease marker, alloisoleucine. The disorder varies in severity and clinical spectrum is quite broad with five recognized clinical variants that have no known association with genotype. The classic presentation occurs in the neonatal period with developmental delay, failure to thrive, and maple syrup or in the cerumen and urine, and can lead to irreversible neurological complications, including stereotypical movements, metabolic decompensation, and death if left untreated. Treatment consists of dietary restriction of BCAAs and close metabolic monitoring. Clinical outcomes are generally good in patients where treatment is initiated early.

The biggest challenge in this patient is the individualized nutrition as consequence of the extremely rare combination of 2 rare metabolic diseases in one person. Zero natural protein diet is mandatory special individualized amino acid supplements. Liver transplantation would be an option in isolated MSUD, but is not beneficial in MMA, therefore a strict dietary regime has to followed live long.

P093-F | Maternal obesity (MO) in sheep alters offspring mitochondrial hepatic biology and redefines the protein acetylation pattern

I. Cardoso*; T. Cunha-Oliveira*; S. Pinho*; C.H. Cavallaro*; I. Baldeiras*,†; S.P. Pereira*,‡; S. Ford§; P.W. Nathanielsz§; P.J. Oliveira*

*MitoXT - Center For Neuroscience And Cell Biology, University of Coimbra, UC-Biotech, Biocant Park, Cantanhede, Portugal; †Neurological Clinic, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; ‡Research Centre in Physical Activity Health and Leisure, Faculty of Sports, University of Porto, Porto, Portugal; §Department of Animal Science, University of Wyoming, Laramie, USA

Background: Changes in the uterine nutritional environment in maternal obesity (MO) lead to alterations in fetal hepatic metabolism, predisposing offspring (F1) to later-life metabolic diseases. In the fetus, the PaO2 of blood supplying the left (L) liver lobe is higher than the right lobe resulting in distinct metabolic profiles. Since mitochondrial bioenergetics plays a crucial role in hepatic metabolism and function, we hypothesize that MO programs F1 fetal liver mitochondrial profile in a lobe-dependent manner.

Methods: Rambouillet obese ewes (MO, $n = 10$) ate 150% of feed eaten by controls (C, $n = 8$) from 60 days prior to conception throughout pregnancy. Both fetal livers lobes were removed at 0·9 gestation and tissue or subcellular fractions were used to assess mitochondrial parameters and acetylome profile analysis. Comparison between groups was performed by Mann-Whitney test, significance set at $P < 0{\cdot}05$.

Results: mtDNA copy number increased in the MOF1 R lobe when compared with CF1 R lobe. A decrease in mitochondrial respiratory chain complexes activity was observed, being more evident in the L lobe, except for complex I where the R lobe was more affected. No differences were observed in liver tissue redox state, except for glutathione peroxidase activity which was decreased in the MO L lobe ($P = 0{\cdot}026$). Pyruvate dehydrogenase activity showed a marked tendency for a decrease in the MO R lobe ($P = 0{\cdot}05$). Additionally, protein acetylation pattern was found to be altered, with the absence of a $\approx$ 37 kDa acetylated protein band in the cytosolic fraction of MO R/L lobe.

Conclusions: The observed changes in the activity of key hepatic mitochondrial bioenergetics enzymes, especially in the L lobe, suggest hepatic metabolic changes induced by MO that may have a significant impact on F1 liver function, potentially affecting the predisposition to later-life metabolic diseases.

Funded by FEDER/COMPETE/FCT-Portugal (PTDC/DTP-DES/1082/2014, POCI-01-0145-FEDER-007440, POCI-01-0145-FEDER-016657, SFRH/BPD/116061/2016 and SFRH/BPD/101169/2014), and NIH (R01HD070096-01A1).

P094-F | Genetic variant rs1260326 of GCKR as an indicator of the risk of obesity at early ages

H.M. Pasero*; E.A. Aguilar*; I.E. Salinas*; R. de la Iglesia*; S. Molina*; G. Colmenarejo*; A.R. de Molina*; G. Reglero*,†; V.L. Kohen*

*IMDEA-Food Institute. CEI UAM+CSIC, Madrid, Spain; †Department of Production and Characterization of Novel Foods. Institute of Food Science Research (CIAL) CEI UAM+CSIC, Madrid, Spain

Background: Glucokinase is a key enzyme in the regulation of glucose homeostasis. Different studies have shown an association between the presence of the rs1260326 polymorphism of the glucokinase regulator gene (GCKR), lower insulin resistance and diabetes, but its association in relation to body composition has been less studied. The main objective of this study was to evaluate the association between the presence of this polymorphism and body composition in a child group from public schools throughout Madrid.

Material and methods: Anthropometric data (height, weight, body composition analysis with bioelectrical

impedance (BF511-OMROM Healthcare), tricipital skinfold (TS)) and saliva samples for DNA extraction where collected from 221 schoolchildren. According to the body mass index (BMI), the volunteers were classified based on the IOTF criteria. Genomic DNA was genotyped by Taqman Open Array Genotyping Platform. Adjusted for sex and age logistic and linear regressions were made. The data was analyzed with R Statistical Software 2·15. Genetic analyses were performed using the dominant model and the results found to be significant when $P < 0{\cdot}05$.

Results: The genotype frequencies were 19·46% TT, 49·77% CT and 30·7% CC. Participants carrying one T allele (TT and CT) presented significantly lower values of fat mass than major allele homozygous (CC) (18·78%, 20·46%, 21·87% respectively, beta = −1·44 (−2·81/−0·08), $P = 0{\cdot}038$), of BMI (16·26, 16·87 and 17·54 kg/m^2 respectively, beta = −0·6 (−1·09/−0·1), $P = 0{\cdot}018$) and of TS. The percentage of overweight/obese children was also significantly lower in the carriers of at least one T allele (14% TT, 26·4% CT and 30·9% CC, OR = 0·64 (0·41–1·01), $P = 0{\cdot}049$).

Conclusion: According to the overweight and obesity alarming rates, the search for tools that allow early detection from childhood becomes necessary. The determination of genetic variants could be very useful for early, individualized and more effective primary prevention.

P095-F | Evaluation of HDL cholesterol efflux capacity (CEC) after consumption of an innovative pasta enriched with bioactive components and functional probiotics

E. Favari; E. Cipollari; F. Zimetti; M.P. Adorni; N. Ronda; D. Angelino; F. Bernini; N. Pellegrini

Department of Food and Drug, University of Parma, Parma, Italy

Background: Epidemiological evidence indicate that high intake of whole grain is associated with a reduced risk of cardiovascular and metabolic disease. This study aimed to investigate the effect of consumption of a new functional whole grain on serum HDL-CEC, a metric of HDL functionality to promote reverse cholesterol transport, which has recently emerged as a new marker for cardiovascular risk evaluation.

Material and methods: 40 healthy volunteers were randomly assigned to two treatments such as experimental pasta made with whole-wheat flour enriched in β-glucan from barley and spores of *B. coagulans* GBI-30 and control pasta produced with the same technological process and with the same, but not integral, variety of wheat as the functional one. CEC measurement was performed ex vivo on whole plasma collected from subjects before and after three months of treatment. Individual cholesterol efflux pathways were evaluated by using specific, widely accepted cell-based radio assays.

Results: In our study, despite no change in HDL concentration, we did observe an improvement in ABCG1 CEC after treatment with the innovative pasta. Additionally, in treated subjects, but not in subjects treated with control pasta, ABCG1-mediated CEC inversely correlates with homocysteinemia, an independent risk factor for coronary disease, while a direct significant relation was found with plasmatic folic acid, which is considered a protective factor for cardiovascular disease.

Conclusions: Since HDL-CEC has been suggested as a new biomarker in CVD, our study is relevant to prove that treatment with a functional food modulates HDL functionality. Correlations between plasmatic indices of cardiovascular disease and CEC provide new insight on its role as a biomarker. It can be speculated that consumption of bioactive components within the innovative pasta act simultaneously on the amelioration of subjects inflammatory profile and on HDL functional quality thus "linking" ABCG1 CEC to the levels of such metabolic markers.

P096-F | Metabolic abnormalities in normal weight children: role of fructose and intestinal permeability

A. Nier*; I.B. Maier†; Y. Özel†; I. Bergheim*

**University of Vienna, Vienna, Austria; †University of Hohenheim, Stuttgart, Germany*

Background: By now, overweight and obesity in adults and children have been identified as the main risk factors for the development of metabolic disorders. However, recent studies have shown that normal weight individuals are also frequently affected by metabolic impairments with underlying mechanisms are not yet fully understood. The aim of the present study was to determine if alterations of dietary pattern and subsequent nutritional intake, markers of intestinal permeability or inflammation in normal weight children with metabolic abnormalities differ from normal weight children without metabolic abnormalities.

Materials and methods: In total, 45 normal weight children aged 5–9 years old were included in the study of whom 9 suffered from one or more metabolic abnormalities (hypertension, hypertriglyceridemia, HDL <5 percentile or NAFLD). Anthropometric data, dietary intake and markers of inflammation as well as intestinal permeability were assessed in fasting blood samples.

Results: Neither BMI nor BMI-SDS or BMI-percentiles differed between groups; however children with metabolic abnormalities had a significantly bigger waist circumference (+5 cm). While total caloric and macronutrient intake was similar between groups, mean fructose intake resulting mainly from sugar sweetened beverages and breakfast cereals was significantly higher in children with metabolic abnormalities than in healthy children. Time spent physically active was significantly longer in healthy normal weight children whereas time spent physically inactive was similar between groups. Furthermore, bacterial endotoxin and leptin levels, the latter being used as a marker of visceral adiposity, were significantly higher in plasma of normal weight children with metabolic abnormalities than in healthy normal weight children. Neither TNFα nor sCD14, adiponectin, PAI-1 or IL-6 plasma levels differed between groups.

Conclusion: Our results suggest that metabolic disorders in normal weight children are associated with a high fructose intake and elevated bacterial endotoxin as well as leptin plasma levels, the latter also being discussed to be indicative of visceral adiposity.

P097-F | Association of NBPF3 gene polymorphism with vitamin B6 level among Tatars

A. Syrocheva; E. Valeeva; O. Kravtsova

Kazan Federal University, Kazan, Russian Federation

Introduction: Vitamin B6, also known as pyridoxine, is one of the vitamins that plays an important role in a range of physical and psychological functions. Several GWAS showed the association between vitamin B6 level and NBPF3 gene polymorphism but this data still controversive among worldwide populations.

Aim: To revalidate the association of NBPF3 gene polymorphism (rs4654748) with vitamin B6 level in healthy donors represented by randomized population of Tatar, Russia.

Materials and methods: SNP was genotyped in DNA samples extracted from buccal swabs from 576 non-relative healthy donors of Tatar ethnicity (275 male and 301 female) by real-time PCR using TaqMan probes (Novosibirsk, Russia). Vitamin B6 level (VitB6) was determined by colorimetric method with ferrum chloride (III) in serum of 96 individuals (39 and 57 male and female respectively aged from 18 to 48 years). Statistical analysis was performed by STATISTICA v.10.

Results: No deviation from HWE was detected ($\chi^2 = 0{\cdot}568$, $P = 0{\cdot}451$). The prevalence of CT genotype (45·8%) was determined independent from gender (38·6% and 35·8% for female and male respectively).

VitB6 level for all samples was according to reference level (16·47 ± 1·18 ng/mL, references 8·7–20·2 ng/mL). Slightly lowering VitB6 was detected in men (15·07 ± 1·87 ng/mL) vs women (17·5 ± 0·5 ng/mL) but with no significant difference. Also, we have found a tendency of increasing VitB6 level in older age group but without statistical significance ($r = 0{\cdot}2112$, $P > 0{\cdot}05$).

Furthermore, no significant difference was detected in VitB6 level among different genotype carriers: for CC genotype vitamin B6 level was 16·43 ± 2·2 ng/mL, for CT genotype—16·54 ± 2·09 ng/mL, for TT genotype—16·43 ± 1·94 ng/mL.

Conclusion: Our data indicates that rs4654748 NBPF3 gene polymorphism doesn't play a key role in vitamin B6 level in healthy donors in Volga Tatar population.

This work was supported by Program of Competitive Growth of KFU.

P098-F | Influence of gene polymorphism on vitamin D level among healthy women from Republic of Tatarstan

I. Garifullova*; E. Valeeva*,†; O. Kravtsova†

**Kazan State Medical University, Kazan, Russian Federation; †Kazan Federal University, Kazan, Russian Federation*

Introduction: In recent decades it's shown that circulating vitamin D (25-hydroxy-vitamin D, vitD) plays role not only in canonical activities of calcium homeostasis. Also vitD deficiency has been implicated in a number of chronic diseases including cancer. Several GWAS pointed some genes which polymorphism can be associated with vitD level, however no data presents for the European ancestry population from Republic of Tatarstan.

Aim: Association study of VDR, CYP2R1 and GC (DBP) genes polymorphism with circulation vitD level in healthy women in Tatar population.

Materials and methods: Circulating vitD measured in serum of 124 women (aged from 19 to 52 years) by ELISA. SNP genotyping for rs2228570, rs2060793 and rs2282679 was performed in 384 DNA samples by real-time PCR. Statistical analysis done with packet program SPSS Statistics v.20.

Results: No significant vitD deficiency was detected in studied group (29·93 ± 1·32 ng/mL). Besides vitD level didn't dependent from isolation, age and additional vitD supplement ($P > 0{\cdot}05$). Furthermore, all studied polymorphisms were according with HWE ($P > 0{\cdot}05$): TT genotype carriers of VDR gene polymorphism were

characterized by significant vitD lowering compared to other individuals (26·4 ± 1·2 and 29·3 ± 1·3 ng/mL). However, no significant difference in circulating vitD level was determined between different genotype carrier for CYP2R1 and DBP gene polymorphism ($P > 0·05$).

Conclusion: Our data indicates that VDR gene polymorphism is associated with circulation vitamin D level in healthy women of Tatar ethnicity.

This work was supported by Program of Competitive Growth of KFU.

P099-F | Genome-wide screening of polymorphisms of microRNAs identifies a variant in the mir-3183 newly associated with type-2 diabetes risk in a high cardiovascular risk Mediterranean population: modulation by fat intake

O. Coltell*; E.M. Asensio†; R. Barragán†; J.V. Sorlí†; J.I. González†; O. Portolés†; C. Ortega-Azorín†; C.Q. Lai‡; J.M. Ordovas‡; D. Corella†

*Universitat Jaume I-CIBER Fisiopatología de la Obesidad y Nutrición, Castellon, Spain; †University of Valencia-CIBER Fisiopatología de la Obesidad y Nutrición, Valencia, Spain; ‡Jean Mayer-USDA Human Nutrition Research Center on Aging at Tufts University, Boston, USA

Background: Precision nutrition, among other factors, includes the knowledge and understanding of individual differences in the genomic sequence to provide tailor-made dietary advice. Assessing the effects of variants in noncoding elements, in particular microRNAs, is crucial as a single microRNA can influence the expression patterns of many genes at the same time. Although there are several studies showing associations between circulating levels of microRNAs and type-2 diabetes, the investigation of the effects of single nucleotide polymorphisms (SNPs) in microRNA on type-2 diabetes risk is scarce. Our aim was to analyze the association between microRNA-SNPs, type-2 diabetes risk and the dietary modulation of the top-ranked SNP.

Methods: We studied 1045 high cardiovascular risk participants (46% diabetes) from Valencia, Spain. Genome-wide genotyping was carried out by the Illumina Human OmniExpress Array. For the Identification of SNPs in miRNA-encoding sequences we screened all known human miRNAs report in the miRBase database and extracted the corresponding data from our genome-wide array. Minor allele frequency (MAF), Hardy-Weinberg and linkage disequilibrium rules were applied. We identified dozens of independent microRNA SNPs and analyzed the association with type-2 diabetes at baseline, identifying several microRNA-SNPs. We analyzed the modulation by dietary intake for the top-ranked SNP.

Results: The top-ranked microRNA-SNPs associated (after correction for multiple comparisons) with type-2 diabetes was the rs2663345 (A>C) in chromosome 17, MAF: 0·3, in the miR-3183 (Pre-mir location). In an additive model, the minor allele was associated with lower type 2 diabetes risk at baseline ($P < 0·001$). In a dominant model, homozygous carriers of the mayor allele had increased type-2 diabetes risk both in men (OR: 1·95; 95% CI: 1·28–2·96) and women (OR: 1·45; 95% CI: 1·06–1·98). Fat intake, mainly monounsaturated fat, modulated such association with type-2 diabetes risk.

Conclusions: After genome-wide screening we have identified a novel association of a microRNA SNP with type-2 diabetes that could be modulated by diet.

OBESITY-CIBEROBN

P162-T | Role of ghrelin and glucagon-like peptide-1 in aquaporin-7-induced insulin secretion and triacylglycerol accumulation in rat β-cells

A. Rodríguez*,†; L. Méndez-Giménez*,†; S. Becerril*,†; S. Camões‡; I.V. de Silva‡; C. Rodrigues‡; R. Moncada†,§; V. Valentí†,¶; V. Catalán*,†; J. Gómez-Ambrosi*,†; J.P. Miranda‡; G. Soveral‡; G. Frühbeck*,†,**

*Metabolic Research Laboratory, Clínica Universidad de Navarra, Pamplona, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Spain; ‡Research Institute for Medicines (iMed.ULisboa), Universidade de Lisboa, Lisboa, Portugal; §Department of Anesthesia, Clínica Universidad de Navarra, Pamplona, Spain; ¶Department of Surgery, Clínica Universidad de Navarra, Pamplona, Spain; **Department of Endocrinology & Nutrition, Clínica Universidad de Navarra, Pamplona, Spain

Background: The main goal of the present study was to analyse the role of ghrelin and glucagon-like peptide 1 (GLP-1) in the improvement of pancreatic function and steatosis after the restrictive bariatric surgery procedure sleeve gastrectomy in diet-induced obese rats through the regulation of aquaporin-7 (AQP7), the main glycerol channel in β-cells.

Material and methods: Male Wistar obese rats ($n = 125$) were subjected to surgical (sham surgery and sleeve gastrectomy) or dietary (pair-fed to the amount of food eaten by sleeve-gastrectomized animals) interventions. The effect of acylated and desacyl ghrelin as well as GLP-1 on insulin secretion, triacylglycerol accumulation and AQP7 expression was evaluated in vitro in rat RIN-m5F β-cells.

Results: Sleeve gastrectomy decreased pancreatic β-cell apoptosis, steatosis and insulin secretion (all $P < 0·05$). Lower ($P < 0·001$) circulating total ghrelin and a tendency towards higher ($P = 0·155$) GLP-1 concentrations were also found after bariatric surgery. Acylated and desacyl ghrelin

increased (both $P < 0{\cdot}05$) intracellular triacylglycerol accumulation, whereas GLP-1 induced insulin release ($P < 0{\cdot}05$) in the RIN-m5F β-cell line. An upregulation of pancreatic AQP7 mRNA and protein (both $P < 0{\cdot}05$) was observed in rats submitted to sleeve gastrectomy compared to sham-operated animals. Interestingly, ghrelin and GLP-1 repressed AQP7 mRNA and protein (all $P < 0{\cdot}05$) expression in RIN-m5F β-cells. AQP7 protein was negatively correlated with triacylglycerol content in acylated ghrelin-stimulated cells and with insulin release in GLP-1-treated β-cells.

Conclusions: AQP7 upregulation in β-cells after sleeve gastrectomy partially contributes to the improvement of pancreatic steatosis and insulin secretion by increasing intracellular glycerol utilized for GLP-1-induced insulin release rather than for triacylglycerol biosynthesis triggered by ghrelin.

Conflict of interest: The authors declare that they have no conflict of interest.

Funding: Supported by FIS-FEDER (FIS PI16/00221 and PI16/01217) and CIBEROBN from the Instituto de Salud Carlos III.

P163-T | Sleeve gastrectomy and Roux-en-Y gastric bypass improve hepatic inflammation in diet-induced obese rats

S. Ezquerro[*,†]; L. Méndez-Giménez[*,†]; S. Becerril[*,†]; R. Moncada[†,‡]; V. Valentí[†,§]; V. Catalán[*,†]; J. Gómez-Ambrosi[*,†]; G. Frühbeck[*,†,¶]; A. Rodríguez[*,†]

[*]Metabolic Research Laboratory, Clinica Universidad De Navarra, Pamplona, Spain; [†]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; [‡]Department of Anesthesia, Clínica Universidad de Navarra, Pamplona, Spain; [§]Department of Surgery, Clínica Universidad de Navarra, Pamplona, Spain; [¶]Department of Endocrinology & Nutrition, Clínica Universidad de Navarra, Pamplona, Spain

Background: The progression from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) is explained by the accumulation of triacylglycerols in hepatocytes, which increases their susceptibility to secondary injuries, such as inflammation. We aimed to evaluate the impact of two bariatric surgery procedures, namely sleeve gastrectomy and Roux-en-Y gastric bypass (RYGB) in the hepatic inflammation of diet-induced obese (DIO) rats.

Material and methods: Four-week-old male Wistar rats ($n = 129$) were fed a normal diet or a high-fat diet for 4 months. DIO rats were subjected to surgical [sham surgery, sleeve gastrectomy and RYGB] or dietary interventions [pair-fed to the amount of food eaten by sleeve gastrectomy or RYGB group]. Hepatic inflammation was evaluated by the analysis of the gene expression of proinflammatory factors Tnf, Il6 and Crp by real-time PCR and the activation of proinflammatory c-Jun N-terminal kinase (JNK) signalling pathway by Western-blot.

Results: An improvement in hepatic function and steatosis was observed in DIO rats four weeks after sleeve gastrectomy or RYGB compared to sham-operated and pair-fed groups. Obesity was associated with higher JNK-P/JNK ratio and an upregulation ($P < 0{\cdot}05$) of proinflammatory cytokines Tnf, Il6 as well as a tendency towards an increase in Crp in the liver. Interestingly, both sleeve gastrectomy and RYGB decreased ($P < 0{\cdot}05$) the expression of inflammatory mediators Tnf (73% and 79%), Il6 (75% and 81%) and Crp (48% and 70%) as well as the JNK-P/JNK ratio (48% and 58%) compared to sham-operated groups.

Conclusions: Our results show that sleeve gastrectomy and, to a higher extent, RYGB ameliorate hepatic inflammation by reducing the expression of proinflammatory mediators and the activation of JNK.

Conflict of interest: The authors declare that they not have conflict of interest.

Funding: Supported by FIS-FEDER (PI13/01430, PI16/00221 and PI16/01217) and CIBEROBN from the Instituto de Salud Carlos III.

P164-T | Role of leptin in inflammation and extracellular remodelling of adipose tissue via nitric oxide

S. Becerril[*,†,‡]; A. Rodríguez[*,†,‡]; V. Catalán[*,†,‡]; L. Méndez-Giménez[*,†,‡]; B. Ramírez[*,†,‡]; N. Sainz[*]; M. Llorente[§]; X. Unamuno[§]; J. Gómez-Ambrosi[*,†,‡]; G. Frühbeck[*,†,‡,¶]

[*]Metabolic Research Laboratory, Clínica Universidad de Navarra, Pamplona, Spain; [†]CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Spain; [‡]Obesity and Adipobiology Group, Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain; [§]Medical Engineering Laboratory, University of Navarra, Pamplona, Spain; [¶]Department of Endocrinology & Nutrition, Clínica Universidad de Navarra, Pamplona, Spain

Background: The adipose tissue (AT) extracellular matrix (ECM) remodelling in obesity involves matrix synthesis and degradation, with increased deposition of ECM proteins including tenascin C (TNC). Our aim was to analyze the influence of iNOS deletion in inflammation and ECM remodelling of AT in ob/ob mice because a functional relationship between both leptin and iNOS has been defined.

Material and methods: A double knockout (DBKO) mouse simultaneously lacking the ob and the iNOS genes was generated and the expression of molecules involved in inflammation and ECM remodelling were analyzed in AT. Moreover, leptin-deficient mice were classified in three groups: control, leptin-treated (1 mg kg[sup]-1[/sup] day [sup]-1[/sup]) and pair-fed.

Results: The absence of the ob gene increased inflammation and fibrosis in AT. As expected, leptin treatment corrected the obese phenotype of ob/ob mice. iNOS deletion in ob/ob mice improved insulin sensitivity, AT inflammation and ECM remodelling, as evidenced by lower AT macrophage infiltration ($P < 0{\cdot}01$) and collagen deposition ($P < 0{\cdot}01$), a downregulation of the proinflammatory and profibrogenic genes Tnf ($P < 0{\cdot}01$), Emr1 ($P < 0{\cdot}01$), Hif1a ($P < 0{\cdot}01$), Col6a1 ($P < 0{\cdot}01$), Col6a3 ($P < 0{\cdot}01$) and Tnc ($P < 0{\cdot}05$), as well as lower ($P < 0{\cdot}05$) circulating TNC levels. Moreover, leptin upregulated ($P < 0{\cdot}05$) TNC expression and release via NO-dependent mechanisms in 3T3-L1 adipocytes.

Conclusion: Ablation of iNOS improved AT inflammation and ECM remodelling-related genes of ob/ob mice by decreasing fibrosis and metabolic dysfunction. The synthesis and release of profibrogenic and proinflammatory TNC depend on iNOS activation induced by leptin, suggesting an important role of this alarmin in the development of AT inflammation and fibrosis in the obese state.

Conflict of interest: None disclosed.

Funding: Supported by fondos FIS-FEDER (PI16/00221 and PI16/01217) and CIBEROBN of the Instituto de Salud Carlos III and by the Department of Health of Gobierno de Navarra (61/2014).

P165-T | Increased levels of the extracellular matrix proteins osteopontin, chitinase-3 like-1 and tenascin C in obese patients with colon cancer

V. Catalán*,†; J. Gómez-Ambrosi*,†; A. Rodríguez*,†; B. Ramírez*,†; M. Izaguirre*; J.L. Hernández-Lizoain‡; J. Baixauli‡; V. Valentí†,‡; R. Moncada†,§; C. Silva†,¶; J. Salvador†,¶; G. Frühbeck*,†,¶

**Metabolic Research Laboratory, Clínica Universidad de Navarra, Pamplona, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Pamplona, Spain; ‡Department of Surgery, Clínica Universidad de Navarra, Pamplona, Spain; §Department of Anesthesia, Clínica Universidad de Navarra, Pamplona, Spain; ¶Department of Endocrinology, Clínica Universidad de Navarra, Pamplona, Spain*

Background: A robust link between excess adipose tissue and increased incidence of colon cancer (CC) is now evident. Detailed mechanisms that mediate the obesity-driven effect on cancer development in humans are still poorly understood but chronic inflammation and extracellular matrix (ECM) remodelling have been proposed as plausible mechanisms in cancer progression. The aim of this study was to investigate whether obesity can influence circulating levels of inflammation-related ECM proteins in patients with CC, promoting a microenvironment favourable for tumour growth.

Materials and methods: Circulating concentrations of the ECM proteins osteopontin (OPN), chitinase-3-like protein 1 (YKL-40), tenascin C (TNC) and lipocalin-2 (LCN-2) were determined by ELISA in a cohort of 79 subjects [(47 males/32 females), 26 lean (LN) and 53 obese (OB)]. Enrolled subjects were further subclassified according to the established diagnostic protocol for CC (44 without CC and 35 with CC).

Results: Significant differences in circulating OPN, YKL-40 and TNC concentrations between the experimental groups were observed, being significantly increased due to obesity ($P < 0{\cdot}01$) and CC ($P < 0{\cdot}05$). LCN-2 levels were affected by obesity ($P < 0{\cdot}05$), but no differences were detected regarding the presence or not of CC. No differences ($P > 0{\cdot}05$) between male and female subjects were detected for OPN, TNC and LCN-2 levels. However, circulating concentrations of YKL-40 were significantly higher ($P = 0{\cdot}026$) in males compared with females. A positive association ($P < 0{\cdot}05$) with different inflammatory markers was also detected.

Conclusions: The largely increased circulating levels of the pro-inflammatory ECM proteins OPN, YKL-40 and TNC found in obese patients with CC provides evidence for the important role of these proteins on CC development. Therefore, an influence of obesity in CC development via these ECM proteins can be suggested representing promising diagnostic markers or target molecules for therapeutics.

1. Conflict of interest: None disclosed. 2. Supported by FIS-FEDER (PI14/00950 and PI16/01217) and CIBEROBN.

P166-T | Altered leptin-adiponectin axis in the metabolic syndrome denote a dysfunctional adipose tissue associated with inflammation and oxidative stress in mice and humans

J. Gómez-Ambrosi*,†,‡; V. Catalán*,†,‡; A. Rodríguez*,†,‡; B. Ramírez*,†,‡; S. Becerril*,†,‡; J. Salvador*,†,§; P. Portincasa¶; I. Colina‡,**; G. Frühbeck*,†,‡,§

**Metabolic Research Lab, Clínica Universidad de Navarra, Pamplona, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Pamplona, Spain; ‡Obesity and Adipobiology Group, Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain; §Department of Endocrinology & Nutrition, Clínica Universidad de Navarra, Pamplona, Spain; ¶Clinica Medica "A. Murri", Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, Policlinico Hospital, Bari, Italy; **Department of Internal Medicine, Clínica Universidad de Navarra, Pamplona, Spain*

Background: Obesity and the metabolic syndrome (MS) are characterized by elevated circulating leptin concentrations, in parallel to a decrease in blood levels of adiponectin. Our aim was to assess the effect of leptin replacement on adiponectin levels and expression in leptin-deficient ob/ob mice. Furthermore, to study whether the leptin-adiponectin axis may have a pathophysiological role in the increased systemic inflammation and oxidative stress in ob/ob mice and patients with the MS.

Material and methods: Twelve-week-old male mice were treated with either saline or leptin for 18 days. Moreover, leptin, adiponectin, and markers of inflammation and oxidative stress were measured in 140 Caucasian subjects 60 with and 80 without the MS.

Results: Leptin replacement restored values of adiponectin ($P < 0{\cdot}001$), reduced circulating 8-isoprostane and SAA levels ($P < 0{\cdot}05$), and significantly downregulated the increased gene expression of osteopontin ($P < 0{\cdot}05$), Saa3 ($P < 0{\cdot}05$), Cd68 ($P < 0{\cdot}01$), Il6 ($P < 0{\cdot}01$) and NADPH oxidase ($P < 0{\cdot}01$) in the perirenal WAT and osteopontin ($P < 0{\cdot}05$) in the liver of ob/ob mice. In cultured adipocytes from ob/ob mice, leptin increased ($P < 0{\cdot}05$) the mRNA expression and secretion of adiponectin. The ratio adiponectin/leptin was dramatically decreased ($P < 0{\cdot}001$) in subjects with the MS, whereas systemic oxidative stress, as evidenced by levels of TBARS, as well as markers of inflammation such as SAA, CRP and osteopontin were significantly increased ($P < 0{\cdot}05$). Furthermore, the ratio adiponectin/leptin was negatively correlated with SAA concentrations as well as with CRP levels.

Conclusions: Circulating concentrations of adiponectin are positively regulated by leptin and ameliorate obesity-associated oxidative stress and inflammation in mice. A dysfunctional adipose tissue as suggested by a low adiponectin/leptin ratio may contribute to the increased oxidative stress and inflammation in patients with the MS. New cut-offs to estimate obesity- and MS-associated cardiometabolic risk according to the adiponectin/leptin ratio are proposed.

Supported by grants FIS-PI14/00950 and PI16/01217, ISCIII–FEDER, Fundación-Caja-Navarra-20-2014 and, CIBEROBN, Spain.

P167-T | Patients with functional bowel disorder: effectiveness of a diet low in FODMAPs for the treatment of gastrointestinal symptoms

P. Ibañez*; N. Vila†; V. Santesteban*; J.R. Angós‡; M.T. Betes‡; C. Carretero‡; S. de la Riva‡; M. Herraiz‡; C.P. de frías‡; C. Silva§; M.A. Margall†

**Area of Nutrition, Department of Endocrinology and Nutrition, Clinic of University of Navarra, CIBEROBN, IdiSNA, Pamplona, Spain; †Area of Nutrition, Department of Endocrinology and Nutrition, Clinic of University of Navarra, IdiSNA, Pamplona, Spain; ‡Department of Gastroenterology, Clinic of University of Navarra, Pamplona, Spain; §Department of Endocrinology and Nutrition, Clinic of University of Navarra, CIBEROBN, Pamplona, Spain*

Background: Recent studies indicate that the gastrointestinal symptoms presented by patients with functional bowel disorder improve by restricting the intake of short-chain carbohydrates (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols: FODMAPs).

Objectives: (i) Evaluate the efficacy of a diet low in FODMAPs for the improvement of most gastrointestinal symptoms in patients with functional bowel disorder and patients with inflammatory bowel disease. (ii) Examine the foods within each group of carbohydrates (fructans, galactans, lactose, excess of fructose and polyols) that the patients can again tolerate, after following a diet of reintroducing foods with FODMAPs.

Materials and methods: Prospective study of 445 consecutive patients with functional bowel disorder attending the Nutrition Unit of a Spanish university hospital. The symptoms presented were abdominal pain, bloating, wind, diarrhoea and/or constipation. Patients followed for 6–8 weeks a diet low in FODMAPs, analysing in every case the improvement and adherence to the dietary treatment. They subsequently followed a re-introductory diet of nutrients with FODMAPs, evaluating their tolerance to each one of the food items.

Results: Eighty-six percent of the patients showed an improvement in the gastrointestinal symptoms with a high level of adherence to the dietary treatment. After performing the reintroduction diet, 90% of the patients tolerated again wheat, 85% products with lactose and more than 80% legumes, garlic and stone fruits. More than 75% tolerated 2 pieces of low-fructose fruits simultaneously and other fruits and vegetables with high FODMAPs content.

Conclusions: (i) The diet low in FODMAPs is effective in improving the gastrointestinal symptoms of patients with functional bowel disorder. (ii) Greater adherence to the diet is associated with a general improvement in the gastrointestinal symptoms. (iii) After following the re-introductory phase, the majority of patients tolerated again wheat, milk, dairy products with lactose and legumes, as well as a variety of fruits with high FODMAPs content.

P168-T | The reduction of matrix metalloproteinase-8 serum levels after bariatric surgery is associated with leptin drop and predicts diabetes remission

L. Liberale*,†; A. Bonaventura†; F. Carbone†; M. Bertolotto†; P. Contini‡; N. Scopinaro§,¶; G.B. Camerini¶; F.S. Papadia¶; R. Cordera**; G.G. Camici*; F. Dallegri†,**; G.F. Adami§,¶; F. Montecucco†,**,††

**Center for Molecular Cardiology, University of Zurich, Zurich, Switzerland; †First Clinic of Internal Medicine, Department of Internal medicine, University of Genoa, Genoa, Italy; ‡Clinical Immunology, Department of Internal Medicine, University of Genoa, Genoa, Italy; §International Federation of Surgery for Obesity, Genoa, Italy; ¶Department of Surgery, University of Genoa, Genoa, Italy; **Diabetology Unit, Department of Internal Medicine, University of Genoa, Genoa, Italy; ††First Clinic of Internal Medicine, Ospedale Policlinico San Martino, Genoa, Italy*

Background: Bariatric surgery showed to be effective against both morbid obesity and T2DM. Among several explanations, a beneficial modulation of the inflammatory environment has been purposed. We aimed at investigating the post-surgery serum leptin trend, its relationship with neutrophil activity and surgery outcome. A translational approach will be applied by studying leptin effects on neutrophil function in vitro.

Methods: 44 T2DM patients (12 morbid obese [MOB] and 32 non-MOB controls) were enrolled and underwent bariatric surgery at Ospedale Policlinico San Martino, Genoa (Italy). Metabolic and inflammatory parameters were assessed at baseline, one month, one and three years after surgery. In vitro, the effects of leptin on Il-6-induced human neutrophil degranulation and integrin upregulation were assessed.

Results: Despite similar demographic, lipid and glycemic profiles, MOB T2DM patients showed increased serum inflammatory markers level (e.g. C-reactive protein [CRP], fibrinogen, neutrophil-to-lymphocyte ratio [NLR], matrix metalloproteinase [MMP]-8 and leptin) when compared with non-MOB T2DM ones at baseline. In MOB patients, CRP, fibrinogen and MMP-8 concentrations displayed a reduction already 1 month after surgery. At the same time point, leptin serum values were markedly decreased in the overall cohort; interestingly, leptin and MMP-8 drops from baseline to one month after surgery were positively correlated (Δleptin vs ΔMMP8: $r = 0{\cdot}391$, $P = 0{\cdot}025$). ΔMMP8 inversely correlated with glycated hemoglobin concentration at both one- and three-year follow up. At the cut-off point identified by Youden's index analysis (>0 ng/mL), ΔMMP8 predicted complete T2DM remission at 3-year follow-up at both uni- and multivariate model analysis. In vitro, neutrophil pretreatment with leptin increased IL-6-induced MMP-8 release while impairing CD18 up-regulation.

Conclusion: After bariatric surgery, the reduction of leptin serum levels positively impacts on pro-inflammatory MMP8 levels, in particular in MOB T2DM patients. Post-surgery contraction of MMP8 serum levels predicts complete T2DM remission at three-year follow-up.

P169-T | Effects of Roux-en-Y gastric bypass on adiposity and blood pressure in diet-induced obese rats

X. Unamuno*,†; S. Becerril*,‡,§; A. Rodriguez*,‡,§; J.A. Cienfuegos¶; V. Valentí‡,¶; J. Gómez-Ambrosi*,‡,§; B. Ramirez*,‡,§; S. Ezquerro*,‡,§; L. Méndez-Gimenez*; M. Martín**; M.A. Burrell**; V. Catalán*,‡,§; M.R. Elizalde††,‡‡; G. Frühbeck*,‡,§,§§

**Metabolic Research Laboratory, Clinica Universidad de Navarra, Pamplona, Spain; †Medical Engineering Laboratory, University of Navarra, Pamplona, Spain; ‡CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Pamplona, Spain; §Obesity and Adipobiology Group, Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain; ¶Department of Surgery, Clinica Universidad de Navarra, Pamplona, Spain; **Department of Histology and Pathology, University of Navarra, Pamplona, Spain; ††Ceit, San Sebastian, Spain; ‡‡University of Navarra, Tecnun, San Sebastian, Spain; §§Department of Endocrinology & Nutrition, Clinica Universidad de Navarra, Spain*

Background: Roux-en-Y gastric bypass (RYGB) constitutes an effective surgical procedure for the treatment of morbid obesity. The aim of the present study was to establish the effects of RYGB on weight loss and cardiovascular parameters in diet-induced obese (DIO) rats.

Methods: Six-month-old male Wistar DIO rats ($n = 90$) were subjected to different interventions (sham operation, RYGB, or pair-fed to the amount of food eaten by animals submitted to RYGB) and to two dietary patterns [fed ad libitum a normal diet (ND) or a high-fat diet (HFD)]. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure values (MBP) and heart rate (HR) were recorded in conscious, resting animals by non-invasive tail-cuff plethysmography before and 3 weeks after surgical and dietary procedures.

Results: RYGB induced a reduction in body weight ($P < 0{\cdot}001$), whole-body adiposity ($P < 0{\cdot}05$), mainly due to reductions in perirenal ($P < 0{\cdot}05$), subcutaneous ($P < 0{\cdot}0001$) and epididymal ($P < 0{\cdot}05$) fat depots. An improvement of insulin sensitivity was also observed after RYGB, as evidenced by lower glucose levels ($P < 0{\cdot}05$), insulinaemia ($P < 0{\cdot}0001$) and HOMA index ($P < 0{\cdot}0001$) as well as higher QUICKI index ($P < 0{\cdot}05$). RYGB was further associated with an improvement ($P < 0{\cdot}0001$) in the lipid profile as well as with a reduction in serum leptin ($P < 0{\cdot}001$) and ghrelin levels ($P < 0{\cdot}001$). Although no

changes in DBP and MBP were observed in rats submitted to RYGB, a decreased heart weight ($P < 0{\cdot}0001$) and SBP values ($P < 0{\cdot}05$) was found in operated animals.

Conclusion: RYGB exerts beneficial effects on adiposity, metabolic profile and blood pressure values in diet-induced obese rats even following a HFD.

Conflict of interest: None disclosed.

Funding: This work was supported by grants from the Fondo de Investigación Sanitaria-FEDER, Instituto de Salud Carlos III (ISCIII) (PI16/01217) and CIBEROBN, Spain.

P170-T | Decreased TLR3 in hyperplastic adipose tissue, blood and inflamed adipocytes is related to metabolic inflammation

A. Lluch*,†,‡; J. Latorre†,‡,§; J.M. Moreno-Navarrete†,‡,§; M. Sabater†,‡,§; M. Buxó†; J.I. Rodriguez-Hermosa†,¶; J. Girones†,¶; W. Ricart†,‡,§; R. Vilallonga**; R. Simó††; J.M. Fernández-Real†,‡,§; F.J. Ortega†,‡,§

**Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain; †Institut d'Investigació Biomédica de Girona (IdIBGi), Girona, Spain; ‡Department of Diabetes, Endocrinology and Nutrition (UDEN), Hospital of Girona Dr Josep Trueta, Girona, Spain; §CIBEROBN and Instituto de Salud Carlos III (ISCIII), Madrid, Spain; ¶Department of Surgery, Hospital of Girona "Dr Josep Trueta", Girona, Spain; **Servicio de Cirugía General, Unidad de Cirugía Endocrina, Bariátrica y Metabólica, Hospital Universitario Vall d'Hebron, European Center of Excellence (EAC-BS), Barcelona, Spain; ††Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, CIBERDEM and Instituto de Salud Carlos III (ISCIII), Barcelona, Spain*

Background: Obesity is characterized by the immune activation that dampens insulin sensitivity and modifies metabolism. This study explores the impact of different inflammatory/anti-inflammatory paradigms on the expression of toll-like receptors (TLR) in adipose tissue and blood.

Methods: We evaluated the impact of acute surgery stress in vivo and macrophages (MCM) in vitro. Weight loss was chosen as an anti-inflammatory model, so TLR were analyzed in samples collected before and after bariatric surgery. Associations with inflammation and metabolism were analyzed in non-obese and obese subjects, in parallel with expression measures taken in blood, and in ex vivo isolated adipocytes/stromal-vascular cells (SVC). Treatments with an agonist of TLR3 were conducted in human adipocytes.

Results: Surgery stress raised TLR1 and TLR8 in subcutaneous (SAT), and TLR2 in SAT and visceral (VAT) adipose tissue, while decreasing VAT TLR3 and TLR4. MCM led to increased TLR2, and dampened TLR3, TLR4, and TLR5 in adipocytes. The anti-inflammatory impact of weight loss was concomitant with decreased TLR1, TLR3, and TLR8 in SAT. Cross-sectional associations confirmed increased V/SAT TLR1 and TLR8, and decreased TLR3 in obese patients, as compared with non-obese subjects. TLR were predominant in SVC and adipocyte precursor cells, even though the expression of all of them but TLR8 (very low levels) was found in differentiated adipocytes in close association with the inflammatory state of activation. Among SVC, CD14 + macrophages showed increased TLR1, TLR2, and TLR7, but decreased TLR3 mRNA. The opposite patterns of TLR3 in V/SAT, SVC, and inflamed adipocytes were also observed in blood, being TLR3 linked to lymphocyte instead of neutrophil counts. Concomitantly, decreased TLR3 in inflamed adipocytes dampened lipogenesis and the inflammatory response to Poly(I:C).

Conclusions: Variations of TLR expression in blood and hypertrophied fat depots, namely decreased TLR3 in lymphocytes and inflamed adipocytes, are linked to obesity and metabolic inflammation.

P171-T | Adipose tissue and circulating lysozyme in association with obesity-related metabolic disturbances

J. Latorre*,†,‡; J.M. Moreno-Navarrete*,†,‡; F.J. Ortega*,†,‡; M. Sabater*,†,‡; A. Rodríguez§; S. Becerril§; G. Frühbeck§; R. Vilallonga¶; R. Simó**; W. Ricart*,†,‡; J.M. Fernández-Real*,†,‡

**Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IdIBGi), Girona, Spain; †CIBER de la Fisiología de la Obesidad y la Nutrición (CIBERobn), Madrid, Spain; ‡Department of Diabetes, Endocrinology and Nutrition (UDEN), Hospital of Girona Dr Josep Trueta, Girona, Spain; §Laboratorio de Investigación Metabólica, Clínica Universidad de Navarra (IdiSNA), Pamplona, Spain; ¶Servicio de Cirugía General, Unidad de Cirugía Endocrina, Bariátrica y Metabólica, Hospital Universitario Vall d'Hebron, European Center of Excellence (EAC-BS), Barcelona, Spain; **Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, CIBERDEM and Instituto de Salud Carlos III (ISCIII), Barcelona, Spain*

Background: Lysozyme (LYZ) is an antimicrobial enzyme that is expressed in white blood cells. Obesity pathophysiology implies a chronic low-grade inflammation of adipose tissue (AT) associated to macrophages infiltration. The present study aimed to investigate LYZ in AT and plasma according to obesity status, inflammation and insulin resistance.

Methods: LYZ gene expression and circulating levels were cross-sectionally analysed in subcutaneous and visceral AT (real-time quantitative PCR) and in plasma (ELISA) from subjects with a wide range of fatness and insulin resistance. Adipose tissue LYZ gene expression was also analysed after bariatric surgery-induced weight loss (in humans),

after high-fat diet-induced weight gain (in rats), and immediately after surgical procedure.

Results: The amount of LYZ mRNA expression was comparable to lipogenic genes in AT. Its main expression was detected in cells of the stromal vascular fraction, specifically in CD14 + cells. In both subcutaneous and visceral AT, LYZ mRNA was increased concurrently with body mass index ($P < 0{\cdot}0001$), percent fat mass ($P < 0{\cdot}0001$) and obesity-associated metabolic disturbances (fasting serum glucose, fasting triglycerides, HOMA-IR and C-reactive protein). In addition, AT LYZ mRNA was significantly and positively associated with expression of AT inflammation markers, such as tumor necrosis factor alpha, leptin or lipopolysaccharide binding protein, while negatively with markers of adipogenesis. Bariatric surgery-induced weight loss resulted in decreased subcutaneous fat LYZ mRNA (−68·3%, $P < 0{\cdot}0001$) in parallel to reduction of AT inflammation and the improvement of adipogenesis, while high-fat diet-induced weight gain led to increased Lyz2 mRNA expression (141·5%, $P = 0{\cdot}0003$). Bariatric surgery per se acutely increased LYZ expression, both in subcutaneous (124%, $P = 0{\cdot}014$) and visceral (157%, $P = 0{\cdot}012$) AT. Similar to AT LYZ mRNA, plasma LYZ was also increased with obesity and weight gain, and significantly correlated with obesity-associated metabolic disturbances.

Conclusion: Altogether these findings suggest LYZ as a new player in obesity-associated adipose tissue inflammation and dysfunction.

P172-T | Impact of weight loss in a metabolically healthy pediatric population subject to mediterranean diet and physical exercise

M.M. Melero*; C.G. Escribano*; A.V. Candela*; A.V. Pérez*; P.M. Rodriguez*; J.B. Porrez†; A.R.R. García†; A.R. Jaime‡; J.D. Ruíz§; F.J. Tinahones*; R.G. Huelgas*; R.B. López*

*Institute Of Biomedical Research In Malaga (ibima), Regional University Hospital Of Malaga, Málaga, Spain; †University of Malaga, Málaga, Spain, ; ‡School Medical, Medical team orientation north Malaga, Málaga, Spain; §Cartama Estacion Health Center, Málaga, Spain

Background: An obese prepuber population subgroup defined as "metabolically healthy obese" (MHO) presents a favorable metabolic profile despite the adiposity excess. The objectives were modification of the lifestyle after 4 months of intervention in MHO, observe cardiometabolic improvements, increase adherence to mediterranean diet (MD) and physical activity (PA).

Material and methods: We included 121 children (7·9 ± 1·3 years) with a 24·7 ± 3·5 kg/m^2 BMI and ≤1 of criteria: abdominal circumference and blood pressure with percentile ≥90, triglycerides >90 mg/dL, HDL-c < 40 mg/dL and normal glucose. After modification of the lifestyle (MD and PA) for 4 months, the anthropometric measures, blood pressure, glycemic and lipid profiles, adherence to DM and PA were oriented.

Results: After 4 months, 93·3% of participants (63 boys and 58 girls) slept at least 8 h ($P = 0{\cdot}9$ vs baseline). Significant differences on weight, height, BMI and waist circumference were found in all participants. In 38% of children, BMI decreased almost one point. Weight and waist perimeter decreased for 35·5% and 50·4% respectively. Glucidic and lipid profiles decreased, and no changes were observed. On HDL-c levels, a significant decrease in general population and girls was seeing ($P = 0{\cdot}04$ and $P = 0{\cdot}01$ respectively).

The usCRP and IL-6 levels remain unchanged. However, TNF-α levels decreased in all children and boys ($P = 0{\cdot}03$ and $P = 0{\cdot}01$, respectively), was not observed on girl population ($P = 0{\cdot}55$). Moreover, adherence to MD improved almost for 2 points from the beginning of the intervention ($P = 0{\cdot}0001$) in all participants studied.

Conclusions: Weight loss in MHO children with MD and physical exercise improves the glycemic, lipid and inflammatory profiles. Probably greater cardiometabolic benefits would be observed in longer period of time.

Funding Sources: Granted by Institute of Health Carlos III, cofinancial by the European Regional Development Fund-FEDER (Miguel Servet Type 1 program CP15/00028), and University of Málaga (Campus of International Excellence Andalucía Tech).

P173-T | Evaluation of Meissner corpuscles and Merkel cells in diabetic neuropathy

Y. García-Mesa*; J. García-Piqueras*; J.F. Pérez†; R.C. Pérez*; J.A.V. Álvarez*; O. García-Suárez*

*University of Oviedo. Department of Cell Biology and Morphology, Oviedo, Spain; †Complejo Asistencial Universitario de Salamanca, Salamanca, Spain

Background: Currently, the sanitary approach to diabetic neuropathy is based on the prevention of risk factors, but none of them focuses on the study of peripheral nerve involvement at the epidermal and dermal levels that can lead to a polyneuropathy that is difficult to control. Therefore, our research group focused on the study of two mechanosensitive structures, Meissner corpuscles and Merkel cells, both related to fine touch and proprioception. In addition, immunoreaction was observed for the Piezo2 mechanoprotein, the only ion channel directly related to touch

Methodology: Glabrous skin samples from the distal phalanx of the first finger toe of diabetic patients obtained at the Central University Hospital of Asturias, with a comparable age range between 40 and 65 years. Simple immunohistochemistry was used for the detection of specific antibodies, thus the Piezo2 mechanoprotein.

Results: Diabetic neuropathy is accompanied by a decrease in the Meissner and Merkel cells index. In addition, in the corpuscles, both the axon and the lamellar cells present morphological alterations and the distribution of the antigenic pattern is different. The immunoreaction for Piezo2 is practically non-existent in the two types of sensory structures analyzed.

Conclusions: This study demonstrates that diabetic neuropathy produces a decrease in the number of Meissner corpuscles and Merkel cells, as well as a loss of the mechanotransducing properties of both. These results support the interest of the analysis of Meissner corpuscles and Merkel cells obtained by cutaneous biopsy in the diagnosis-evolution of peripheral neuropathy.

P174-T | miR-1976 regulates CD40 expression in adipocytes by binding to its 3′-UTR region

J.A. Martinez*,†,‡; M. Garcia-Lacarte*; G. Aström§; M. Rydén§; F.I. Milagro*,†,‡

**Department of Nutrition, Food Science and Physiology, Centre for Nutrition Research, University of Navarra, Pamplona, Spain; †CIBERobn, Physiopathology of Obesity and Nutrition, Center of Biomedical Research Network, ISCII, Madrid, Spain; ‡IdiSNA, Navarra's Health Research Institute, Pamplona, Spain; §Department of Medicine, Karolinska Institute, c2-94, Karolinska University Hospital Huddinge, Stockholm, Sweden*

Background: miRNAs play a key role in regulating WAT inflammation and obesity. We have previously evidenced that miR-1976 could be a prospective biomarker of response to specific weight-loss diets and might regulate the expression of its predicted target gene CD40. CD40 attenuates obesity-induced insulin resistance whose deficiency is associated with adipose tissue inflammation and insulin resistance. The aim of this study was to elucidate the relationship between miR-1976 and CD40 in adipocytes.

Material and methods: A time course analysis in human preadipocytes was performed to assay the expression of both miR-1976 and CD40 during adipocyte differentiation. For downregulation experiments, preadipocytes isolated from stromal vascular fraction were differentiated into mature adipocytes for 12 days and transfected with either 20/40 nM of miR-1976 mimic, or Negative Control using Hiperfect Reagent. RNA expression was analyzed 48 hour post transfection. To assess miRNA-target interactions, the 3′UTR region of CD40 was cloned downstream of the firefly luciferase gene in the pmiR-GLO Dual-Luciferase miRNA Target Expression Vector. Then, HEK-293T cells were transiently co-transfected with either 0·25 μg of empty pmiR-GLO or pmiR-GLO-CD40-3′UTR, and 7·5 pmol of miR-1976 mimics using Lipofectamine 2000. Firefly and Renilla luciferase activities were evaluated 24 hour after co-transfection using a Dual-Luciferase Reporter Assay.

Results: miR-1976 expression increased during adipocyte differentiation reaching a maximum at day 12. However, CD40 mRNA expression remained unchanged. Transfection with miR-1976 mimic inhibited CD40 expression. Lastly, HEK-293T cells co-transfected with the pmiR-GLO-CD40-3′-UTR construction and miR-1976 showed a significantly reduction in firefly/Renilla activity than controls transfected only with the expression vector, demonstrating that miR-1976 binds to the 3′UTR of CD40.

Conclusion: CD40 is a target gene of miR-1976 whose expression is regulated by the binding of the miRNA to the 3′UTR region. These results suggest that miR-1976 could be implicated in adipose tissue inflammation and insulin resistance processes.

P175-T | The importance of the intrauterine environment on longevity - gestational obesity as prediction factor for reduced lifespan in descendants

D.-E. Comandasu*; B. Virgolici†; M. Mohora†; C. Mehedintu‡; C. Berceanu§; E. Bratila*

**Department of Obstetrics and Gynecology, "Panait Sarbu" Clinical Obstetrics Gynecology Hospital, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; †Department Biochemistry, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; ‡Department of Obstetrics and Gynecology, "Nicolae Malaxa" Clinical Emergency Hospital, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; §Department of Obstetrics and Gynecology, Craiova County Hospital, Craiova University of Medicine and Pharmacy, Craiova, Romania*

Background: Obesity represents a common pregnancy comorbidity causing obstetrical, neonatal and long-term complications. We aim to present the consequences of gestational obesity on lifespan prognosis of the descendants, showing that longevity is highly correlated with the quality of the intrauterine environment.

Materials and methods: We studied the effects of maternal obesity on descendants' health in adulthood using 30 obese female Wistar rats. We induced rats the obesity by high-calorie high-fat diet administered by gavage and bread

them. The pregnant females were then divided into a group receiving normal diet and another one that continued the fat alimentation during gestation.

Results: Obese rat females were followed throughout gestation and sacrificed at term, along with part of their pups, while another part were followed until natural death. We analyzed the secretion of adipokines from maternal blood (leptin and adiponectin), lipid peroxidation levels by malonyl-dialdehyde and glutathione as antioxidant factor from placental homogenates and maternal blood. Low levels of adiponectin and increased of leptin positively correlated with placental lipid peroxidation measured by elevated MDA and low levels of GSH. Placental histology showed dysplastic epithelial and mesoderm cells in the yolk sac, a higher density of inflammatory cells and congested vessels with thrombotic areas and glycogen trophoblast deposits in the fat group. Following the pups resulted from the obese mothers throughout their adulthood, we found that the medium lifespan of the ones from the fat diet group was significantly reduced compared to the normal one (by up to 30%). These rats were more predisposed to accelerate aging and chronic diseases.

Conclusions: Our study confirmed the important correlation between the biochemical and histopathological alterations of the intrauterine environment proven by placental dysfunction and the reduced lifespan of the descendants. Considering that, we suggest that the quality of the intrauterine life has major impact on longevity.

P176-T | The ratio of Erysipelotrichaceae/Alistipes putredinis as a novel biomarker of obesity-associated gut dysbiosis in humans

M. Sabater-Masdeu; M.E. Palomo-Buitrago; F. Comas; J. Latorre; F.J. Ortega; V. Pérez-Brocal; A. Moya; W. Ricart; J.M. Moreno-Navarrete; J.M. Fernández-Real

Institut D'investigació Biomèdica De Girona (IDIBGI), Girona, Spain

Background: We aimed to investigate the relationship of human gut microbiota composition according to obesity because results obtained by previous studies are inconsistent.

Material and methods: We analyse gut metagenomic microbiota composition according to obesity at baseline and after 2 years follow-up (Cohort 1, $N = 27$). Obesity-associated bacterial families would be confirmed in two large independent cohorts of subjects with different degrees of obesity [Cohort 2 ($N = 539$) and Cohort 3 ($N = 108$)] through RT-PCR. In addition, using a food frequency questionnaire in Cohort 3, the possible contribution of diet on gut microbiota according to obesity was also evaluated.

Results: Metagenomic study revealed that Erysipelotrichaceae and Rikenellaceae were the most abundant bacterial families associated with obesity, being Erysipelotrichaceae RA significantly increased and Rikenellaceae decreased in obese participants. In line with this, Erysipelotrichaceae RA was positively correlated with BMI, waist circumference, HOMA-IR and plasma LBP levels, whereas Rikenellaceae RA was negatively correlated with the same parameters. After 2-years follow-up, the percent change of Erysipelotrichaceae RA was positively correlated with the percent change of waist circumference and plasma LBP. Of note, these associations were confirmed using RT-PCR in cohort 2 and cohort 3. We also found that the ratio between Erysipelotrichaceae RA and Alistipes putredinis RA was associated with obesity-associated metabolic disturbances, being as a novel biomarker of obesity in both cohort 2 and cohort 3. In relation to diet, multivariate regression analysis indicated that simple carbohydrates ($\beta = -0{\cdot}269$, $P = 0{\cdot}011$) was an independent contributor to Alistipes putredinis sp RA variance after controlling for BMI, sex, fasting triglycerides and fatty acid 20:5.

Conclusions: Altogether these data point to the ratio between Erysipelotrichaceae/Alistipes putredinis as a novel biomarker of obesity-associated gut dysbiosis in humans.

P177-T | Microcirculatory improvement induced by laparoscopic sleeve gastrectomy is related to insulin sensitivity retrieval

S. Ministrini*; M.A. Ricci*; R. Paltriccia*; M. Boni†; M.T. Paganelli‡; G. Lupattelli*; L. Pasqualini*

**Università Degli Studi Di Perugia, Perugia, Italy; †"S. Giovanni Battista" Hospital, Foligno, Italy; ‡"S. Maria della Misericordia" Hospital, Perugia, Italy*

Aims: microvascular dysfunction is a potential factor explaining the association of obesity, insulin resistance and vascular damage in morbidly obese subjects. The purpose of the study was to evaluate possible determinants of microcirculatory improvement one year after laparoscopic sleeve gastrectomy (LSG) intervention.

Methods: Thirty seven morbidly obese subjects eligible for bariatric surgery were included in the study. Post-Occlusive Reactive Hyperaemia (PORH) of the forearm skin was measured as Area of Hyperaemia (AH) by laser-Doppler flowmetry before LSG and after a one year follow-up.

Results: After intervention, we observed a significant reduction in BMI, HOMA index, HbA1c and a significant increase of AH in all patients after surgery; this change was not significant in patients with HOMA index and HbA1c levels below the median value of our population.

Also, a significant correlation between the increase of AH and the reduction of both HOMA index and HbA1c was observed.

Conclusions: Our study shows that LSG intervention is correlated with a significant improvement in the microvascular function of morbidly obese subjects; this improvement seems to be related to the baseline degree of insulin-resistance and to the retrieval of insulin-sensitivity post-intervention.

P178-T | Morbid obesity and hypertension: the role of peri-renal fat

M.A. Ricci; M. Scavizzi; S. Ministrini; S.D. Vuono; G. Lupattelli
Internal Medicine, University of Perugia, Perugia, Italy

Background: Accumulation of fat in renal sinus and hilum is associated with hypertension development, but currently there are no studies investigating the role of peri-renal fat in morbid obesity. We evaluated the relationship between peri-renal fat and hypertension in a population of morbid obese patients, and the potential variations after sleeve-gastrectomy (SG).

Material and methods: 284 morbid obese patients were included in the study, 126 underwent SG. At baseline and 10–12 months after surgery, we evaluated anthropometric parameters, blood pressure, glycometabolic and lipidic assessment, and performed bioimpedenziometry and ultrasonographic evaluation of visceral fat area, peri-renal fat thickness and flow-mediated dilation of brachial artery (FMD).

Results: The peri-renal fat thickness in hypertensive obese was higher than in non-hypertensive (13·6 ± 4·8 vs 11·6 ± 4·1, $P = .001$). It showed a significant direct correlation with age, waist circumference, BMI, FMD, systolic blood pressure (SBP), insulinemia, HOMA-IR, glycated hemoglobin and creatinine. The independent predictors ($R2 = .129$) of SBP were peri-renal fat thickness ($\beta = .160$, $P = .022$), FMD ($\beta = -222$, $P = .002$) and age ($\beta = .175$, $P = .011$). After surgery, peri-renal fat thickness significantly decreased (from 13 ± 4 mm to 9 ± 4 mm, $P < .001$). In the 89 hypertensive obese patients who underwent SG, we observed a significant decrease of anti-hypertensive medications needed. Sixteen patients suspended therapy.

Conclusions: The peri-renal fat thickness in obese patients could be a valuable tool to define the risk of developing hypertension, providing the clinician with an additional parameter to define those who need a more aggressive treatment and could benefit most from bariatric surgery.

P179-T | Gastric bypass and sleeve gastrectomy generate similar efficacy in increasing plasma hdl-cholesterol despite inducing different short-term BMI reduction

C. Perdomo*; J. Gargallo*; M. Llavero*; M. García*; J. Escalada*; J.G. Ambrosi*,†; V. Valentí*; C. Silva*; G. Frühbeck*,†; S. Javier*,†
**University Clinic of Navarra, Pamplona, Spain; †CIBEROBN, Madrid, Spain*

Background: The aim of our study was to compare the improvement of body composition and laboratory parameters among patients with severe obesity undergoing proximal Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy (SG) over a period of 12 months.

Methods: We retrospectively reviewed 711 patients that underwent bariatric surgery at our center. Metabolic parameters including lipid profile and body composition (BodPod) variables were analyzed at 6 and 12 months postoperatively.

Results: We reviewed 711 patients (70% women), 608 (85·5%) underwent RYGB and 103 (14·5%) SG. After 6 months postoperatively, BMI reduction was different in both groups (RYGB 11·9 ± 3·4 kg/m^2 −27·5% reduction vs SG 10·4 ± 4·1 kg/m^2 −25·6% reduction; $P = 0·008$). At that time, a clear increase in plasma HDL-cholesterol (HDL-C) was observed after both procedures, being numerically higher in patients who underwent SG (RYGB: 1·1 ± 10·6 mg/dL +5·3% increase vs SG: 3·9 ± 11·0 mg/dL +11, 2% increase; $P = 0·25$). At 12 months postoperatively, BMI reduction was similar in both groups (RYGB: 14·7 kg/m^2 ± 4·6 −33·5% reduction vs SG: 13·3 kg/m^2 ± 5·6 −32·9% reduction; $P = 0·156$), as were the increases in HDL-C (RYGB: 9·6 ± 12·1 mg/dL +23·3% vs SG 14·6 ± 9·8 mg/dL +31·8%; $P = 0·11$). Lower baseline values of BMI ($P = 0·03$) and HDL-C ($P = 0·02$) significantly characterized the group of patients with RYGB in whom HDL-C increased. After 6 and 12 months postoperatively, absolute and percentage change of waist circumference, body fat, fasting glycaemia, LDL-cholesterol, triglycerides, HOMA-R values and leptin plasma levels did not show any significant differences between both surgical techniques.

Conclusions: One year after surgery, RYGB and SG are equipotent in improving BMI, body composition, insulin resistance and lipid profile. Both surgical techniques were able to induce a significant and progressive increase in HDL-C, which does not seem to be directly dependent on body weight reduction.

P180-T | Increased inflammation, oxidative stress and mitochondrial respiration in brown adipose tissue from obese mice

L. Herrero*,†; M. Alcalá‡; M. Calderon-Dominguez*,†; M.C. Soler-Vázquez*,†; S. Zagmutt*,†; K. Ibeas*,†; E. Bustos*; P. Ramos‡; N. Casals†,§; D. Serra*,†; M. Viana‡

**Department of Biochemistry and Physiology, School of Pharmacy and Food Sciences, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, Barcelona, Spain; †Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; ‡Facultad de Farmacia, Universidad CEU San Pablo, Madrid, Spain; §Basic Sciences Department, Faculty of Medicine and Health Sciences, Universitat Internacional de Catalunya (UIC), Barcelona, Spain*

Background: Obesity is associated with severe metabolic diseases such as type 2 diabetes, insulin resistance, cardiovascular disease and some forms of cancer. The pathophysiology of obesity-induced metabolic diseases has been strongly related to white adipose tissue (WAT) dysfunction through several mechanisms such as fibrosis, apoptosis, inflammation, ER and oxidative stress. However, little is known of whether these processes are also present in brown adipose tissue (BAT) during obesity, and the potential consequences on mitochondrial activity.

Material and methods: We characterized the BAT of obese and hyperglycemic mice treated with a high-fat diet (HFD) for 20 weeks.

Results: The hypertrophic BAT from obese mice showed no signs of fibrosis nor apoptosis, but higher levels of inflammation, ER stress, ROS generation and antioxidant enzyme activity than the lean counterparts. The response was attenuated compared with obesity-induced WAT derangements, which suggests that BAT is more resistant to the obesity-induced insult. In fact, mitochondrial respiration in BAT from obese mice was enhanced, with a 2-fold increase in basal oxygen consumption, through the upregulation of complex III of the electron transport chain and UCP1.

Conclusions: Our results show that obesity is accompanied by an increase in BAT mitochondrial activity, inflammation and oxidative damage.

P181-T | Assessment of the nutritional status in junior school children from the city of Kazan

N. Stepanova; E. Valeeva

Kazan Federal University (KFU), Kazan, Russian Federation

According to the WHO data, the obesity level in children and adolescents increased tenfold for the last four decades, having reached 124 million in the year of 2016. Because of the revealed health risks and considerable increase in morbidity, obesity is considered to be one of the major global healthcare problems.

Physical development of 642 schoolchildren aged 7–10 years old from the city of Kazan was assessed according to the WHO standards (2006) with application of the WHO program AnthroPlus (2009) and determination of the Z-score values: WAZ (Weight-for-Age Z-score); HAZ (Height-for-Age Z-score); BAZ (BMI-for-Age Z-score). The study was supplemented with the data of the parents' questionnaire on taking meals in the evening and at weekends.

The assessment of nutritional status according to the WAZ criterion showed that in the study population children with normal body weight made (from −2 SD to +2 SD) 82·6%; the overweight (> +2) was revealed in 11·2% of children, herewith the values exceeded +3 SD in 14 persons, +4 SD in 3 persons. While assessing the nutritional status (BAZ) it was determined that the harmonic physical development (from −2 to +1 SD) was observed in 67·2% children. The overweight was determined in 19·2% of children (from +1 to +2 SD), obesity—in 11·5% (BAZ > +2 SD). Gender analysis showed that 18·8% of girls and 19·8% of boys had overweight and obesity—7·4% and 15·9% correspondingly. Analysis of the questionnaire details showed that the increase of daily food volume in children with overweight was caused by the failure to observe a dietary pattern: increase of the portion size, frequent snacks between the main meals, visiting fast food cafes on the weekend.

This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities 19.9777.2017/8.9

P182-T | Weight loss trajectories in bariatric surgery patients and psychopathological correlates

Z. Agüera*,†; A. García-Ruiz-de-Gordejuela‡; R. Granero*,§; N. Vilarrasa¶, **; M.M.-G. de Bernabé††; I. Sánchez†; S. Jiménez-Murcia*,†,‡‡; F. Fernández-Aranda*,†,‡‡

**CIBER de Fisopatología de la Obesidad Y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; †Eating Disorders Unit, University Hospital of Bellvitge-IDIBELL, Barcelona, Spain; ‡Bariatric and Metabolic Surgery Unit, Service of General and Gastrointestinal Surgery, University Hospital of Bellvitge-IDIBELL, Barcelona, Spain; §Department of Psychobiology and Methodology, Autonomous University of Barcelona, Barcelona, Spain; ¶Department of Endocrinology and Nutrition, University Hospital of Bellvitge-IDIBELL, Barcelona, Spain; **CIBERDEM-CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III, Madrid, Spain; ††Dietetics and Nutrition Unit, University Hospital of Bellvitge, Barcelona, Spain; ‡‡Clinical Sciences Department, School of Medicine, University of Barcelona, Barcelona, Spain*

Background: Several studies have identified weight loss trajectories after bariatric surgery (BS), and have assessed specific techniques of BS. But no study to date, to our knowledge, has sought to compare the empirical trajectories of BMI after BS nor has sought to identify the main psychopathological and personality predictors of BMI trajectories. As such, this study aimed to explore the empirical trajectories of BMI one-year following BS, and to identify the risk factors for each trajectory, which could be useful in developing more efficient interventions for these patients.

Material and methods: This study included 115 severely obese patients who underwent bariatric surgery. Assessment included metabolic variables, psychopathological and personality measures.

Results: Growth-mixture-modeling identified four separated trajectories for the percentage of total weight loss course-shape (namely T1 "good-fast"; T2 "good "; T3 "low"; and T4 "low-slow"). After adjusting for BS subtype and metabolic baseline state, T1 and T2 registered less eating and general psychopathology. T1 was characterized by the lowest scores in novelty seeking and self-transcendence, whereas T4 was defined by the highest novelty seeking and the lowest persistence.

Conclusions: Our findings suggest that psychological state prior to BS is predictive of BMI trajectories during the 12 months following BS. Therefore, some strategies could potentially enhance results in these patients. For example, strategies to cope with middle-long term goals and specific personality traits, such as techniques for decreasing impulsivity, or enhancing persistence, self-directedness and cooperativeness could be recommended before BS. In this line, the development of a temperament and character-focused treatment would be useful in these patients.

P183-T | Features of eating behavior in adolescents

E. Valeeva*; N. Stepanova*; N. Yusupova†; L. Khayrullina†

**Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russian Federation; †KSMA-branch of the State Public Health Service of the Russian Federation Ministry of Health of the Russian Federation, Kazan, Russian Federation*

Experts of the World Health Organization view obesity as an epidemic. The purpose of the study was to study the nutritional behavior of schoolchildren in adolescence in Kazan. The method of questionnaire survey. We conducted a survey of 242 schoolchildren aged 10–15 years. The majority of respondents, the main meal by volume and calorie content is in the evening. According to the results of the survey, only 52% of teenagers include fresh vegetables and fruits in their diets daily. Among meat products, meat (poultry) is regularly consumed 62·5–67·5%. Sausages in the diet are present in 59·8% of respondents. Only 48·5% of schoolchildren eat fish. Every third respondent visits the fast food network once a week, and the main product of consumption is French fries. In the diet of adolescents, the carbohydrate component (national cuisine) predominates at the expense of flour and confectionery products 65·4%, so regularly eat sweets and chocolate 47·1–65·3%. Regularly extra salt food 49·2% of schoolchildren. A positive relationship between age and the presence of pain in the stomach ($r = 0{\cdot}46$), difficulty with falling asleep ($r = 0{\cdot}50$), headaches ($r = 0{\cdot}51$) was determined. To the senior classes the frequency of complaints among students increases. Thus, the availability of food and a variety of delicious food, the national characteristics lead to overeating and, as a result, an increase in body weight. Source of financing "This work was funded by the subsidy allocated to the Kazan Federal University for the state assignment in the sphere of scientific activities". State assignment 19.9777.2017/8.9

P184-T | Results of dietary studies in junior schoolchildren

N. Stepanova*; N. Yusupova†; E. Valeeva*; L. Khairullina†

**Kazan Federal University (KFU), Kazan, Russian Federation; †Kazan State Medical Academy – branch FGBOU DPO of the Russian Medical Academy of Postgraduate, Kazan, Russian Federation*

The problem of overweight and obesity, which was previously considered to be peculiar only to the countries with high income, becomes widespread in urban conditions in particular. The cause of overweight and obesity in children is global shift of nutrition towards consumption of foods with high content of fats, sugar and low content of vitamins, minerals and other nutrients.

Nutrition of 650 schoolchildren aged 7–10 years old from the city of Kazan was studied according to the following indicators: daily food package, chemical composition, the parents' questionnaire survey. The assessment of chemical composition of diets was carried out on the computer program "Analysis of the human nutritional status". It was found out that children received less such foods as meat and meat products (67% of Recommended Dietary Allowances (RDA)), milk and dairy products (72·2% of RDA), fish and sea products (35·0% of RDA), eggs (80·4% of RDA) than they were due. The level of cereals and pasts consumption exceeded RDA by 60·0%. Vegetables and fruit made 92·5% of RDA in daily intake with the children nutrition. It was noted that the nutrition of junior schoolchildren was monotonous, most frequently they ate

pasta, poultry, sausage products and sweets. The dietary energy value made 2154 ± 26 kcal/day (the standard intake being 2100 kcal/day. A carbohydrate-and-fat eating pattern was identified in schoolchildren. The nutrition was imbalanced, the ratio of basic nutrients in daily diets made 1:1·5:6·2 (the standard ratio being—1:1·1:4·8). Nutritional studies of schoolchildren showed a poor level of both school catering, and home catering, low level of parents' knowledge about the principles of healthy nutrition, this fact being the risk factor of overweight in children.

This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities 19.9777.2017/8.9

P185-T | Fibroblast growth factor-21 as a dual hepatokine/adipokine in the control of lipid metabolism

F. Villarroya

University of Barcelona, Barcelona, Spain

Fibroblast growth factor-21 (FGF21) is a hormonal factor with strong anti-diabetic effects. It is released mainly by the liver (hepatokine). Hepatic expression of FGF21 is induced in response to physiological or pathological lipid challenges due to the activation of the FGF21 gene transcription by PPAR-alpha. Moreover, several hepatic insults (e.g. ER stress) cause also a strong induction of hepatic FGF21 expression and release. In fact, FGF21 in blood is increased in obesity, type II diabetes and other metabolic pathological conditions, being a biomarker of hepatic stress and NAFLD in human patients. There are indications that obesity is an FGF21-resistant stage, and metabolic insults eliciting increased FGF21 expression in obesity cause a reciprocal repression of the expression of beta-Klotho, the key co-receptor mediating cellular FGF21 responsiveness. Experimental gain-of-function (FGF21 treatment) and loss-of-function (FGF21-null mice) indicate protective effects of FGF21 on hepatic steatosis. However, FGF21 behaves also as a brown adipokine. Thus, thermal stress leads to a strong secretion of FGF21 by brown fat whereas hepatic FGF21 is repressed and a minor induction of blood FGF21 occurs. White adipose tissue is a target of FGF21 whereas secretion of FGF21 by white fat appears to have mostly an autocrine role, with a minor relevance in humans. The cross-talk of FGF21 regulation in liver and adipose tissues appears as a key pathophysiological event in relation to lipid homeostasis.

P186-T | Oncostatin m is secreted by immune cells and overexpressed in patients with obesity and hyperglycemia: potential role in the development of type 2 diabetes

D. Sanchez-infantes*; Siri.D. Taxeras*; I. Piquer-Garcia*; S. Rodríguez-Fernández*; A. Gavaldà-Navarro[†,‡]; S. Pellitero*[,§]; R. Puig*; E. Martínez*; J. Tarascó*; C. Higueras*; P. Malagón*; F. Villarroya[†,‡]; M. Vives-Pi*; Josep.A. Villena[¶]; M. Puig-Domingo*[,§]

**Institut d'Investigació Germans Trias I Pujol (IGTP), Badalona (Barcelona), Spain; [†]Department of Biochemistry and Molecular Biomedicine. Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, Barcelona, Spain; [‡]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Madrid, Spain; [§]CIBER Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain; [¶]Laboratory of Metabolism and Obesity, Vall d'Hebron Research Institute, Barcelona, Spain*

Background: Obesity can lead type 2 diabetes (T2D), however there are patients with obesity who present euglycemia. The mechanisms by which T2D appears have not been fully elucidated. Oncostatin m (OSM) is a proinflammatory cytokine, member of the IL-6 family, which is increased in obesity in mice and humans, and impairs browning in mice. Here, we aim at evaluating the potential role of OSM in the development of T2D in patients with obesity.

Material and methods: A cohort of 25 patients across a range of BMI (24–60 kg/m^2) were recruited for this study. Patients were classified in 3 groups according to clinical data: (i) Healthy normal-weight controls; (ii) Normoglycemic obesity (fasting glycemia<100 mg/dL); (iii) Hyperglycemic obesity (fasting glycemia>100 mg/dL). Subcutaneous white adipose tissue (sWAT) was collected for RNA analysis. To elucidate the main source of OSM, stromal-vascular fraction (SVF) and adipocytes were isolated. T lymphocytes were also magnetically purified from the SVF to determine gene expression of OSM and its receptor.

Results: OSM mRNA levels were increased in sWAT from patients with obesity compared to healthy controls. Moreover, we observed that OSM mRNA expression was increased in patients with 'hyperglycemic obesity' compared to those who had euglycemia (P = 0·03). A direct correlation was found between OSM gene expression and insulin, HOMA-IR and triglyceride levels.

Moreover, we identified T lymphocytes as the main source of OSM in patients with obesity. In addition, the OSM receptor was detected in SVF and mature adipocytes, and T lymphocytes also showed the highest levels in comparison to other fractions.

Conclusions: Considering the higher levels of OSM found in WAT of obese patients with hyperglycemia, we propose

that this cytokine is involved in the development of insulin resistance. Therefore, OSM might be a novel target molecule for the prevention/treatment of T2D associated with obesity.

P187-T | Surgical complications, weight loss and comorbidity resolution in super obese patients following bariatric surgery

C. Tuero*; L. Granero*; F. Rotellar*; R. Moncada†; M. Landecho‡; C. Silva§; F. Lapuente¶; P.M. Ortega¶; J. Salvador§; G. Frühbeck§; V. Valenti*

**Department of Surgery, Clinica Universidad de Navarra, Pamplona, Spain; †Department of Anesthesiology, Clinica Universidad de Navarra, Pamplona, Spain; ‡Department of internal medicine, Clinica Universidad de Navarra, Pamplona, Spain; §Department of Endocrinology & Nutrition, Clinica Universidad de Navarra, CIBEROBN, IdiSNA, Pamplona, Spain; ¶Department of Surgery, Clinica Universidad de Navarra, Madrid, Spain*

Background: In the last decades there has been a rise in the prevalence of super obese (SO) patients with a BMI over 50 kg/m^2. This group of patients usually have more associated comorbidities representing a higher surgical risk. The aim of our study was to analyse the safety profile and effectiveness of bariatric surgery in SO patients in our hospital.

Material and methods: From January 2010 to December 2015, 121 patients underwent laparoscopic gastric bypass or laparoscopic sleeve gastrectomy; 43 patients (35·5%) were SO. Weight loss and body composition were evaluated at 1 and 6 months as well as at 1, 2, 3 and 5 years. We also measured the percentage of resolution of comorbidities as well as the long and short-term surgical complications.

Results: Five cases exhibited intraluminal bleeding, taking place in only one of the SO subjects. We performed three re-interventions, two in the SO group. Regarding long-term complications in both groups, only incisional hernia was higher in SO. Mortality rate was 0%. Median age in the SO was 40 years with a mean BMI at the time of surgery of 53·6 kg/m^2. Percentage of total weight loss was 29·2%, 29·8%, 27·6% and 27·6% at 1, 2, 3 and 5 years, respectively. Regarding body composition, there was a reduction of body fat of 18·8% and 18·0% at 1 and 2 years, respectively. With regard to comorbidities, the rate of resolution at 5 years in SO patients was 82% for type 2 diabetes mellitus, 79% for obstructive sleep apnea, 72% for hypertension and 69% for joint disease.

Conclusions: In our hospital, bariatric surgery in super obese patients is a safe and effective treatment with low complications and a high resolution rate of comorbidities.

P188-T | Bariatric surgery in elderly obese patients is safe and effective

C. Tuero*; L. Granero*; P. Ahechu*; S. Esteban*; J. Lujan*; F. Rotellar*; J.L. Hernandez*; A. Zugasti†; E. Petrina†; M. de la Higuera‡; G. Frühbeck§; V. Valenti*

**Department of Surgery, Clinica Universidad de Navarra, Pamplona, Spain; †Department of Endocrinology & Nutrition, Hospital de Navarra, Pamplona, Spain; ‡Department of Endocrinology & Nutrition, Clinica Universidad de Navarra, Madrid, Spain; §Department of Endocrinology & Nutrition, Clinica Universidad de Navarra, CIBEROBN, IdiSNA, Pamplona, Spain*

Background: In the last decades, there has been a rise in the prevalence of obese patients. There has also been an increase in life expectancy leading to more elderly obese people (EOP). Bariatric surgery has demonstrated good results in obese patients between 18 and 60 years. The aim of our study was to analyse outcomes of patients older than 60 years as compared to those under this age limit at our institution.

Material and methods: We performed a retrospective study with 121 patients who underwent bariatric surgery between January 2010 and December 2015. We compared both age groups and analysed long-term changes in body composition, complications and comorbidity resolution.

Results: Almost 10% of our cohort were EOP with a mean age of 62 years. Mortality rate was 0% in both groups. No surgical complications in the short- and long-term follow-up in EOP were observed. Mean BMI at the time of surgery was 43·7 kg/m^2. Mean BMI loss in the EOP was 24·0%, 21·5% and 21·6% at 1, 3 and 5 years, respectively. Excess BMI loss in the elderly group was 57·2%, 49·6% and 48·2% at 1, 3 and 5 years, respectively. Percentages of comorbidity resolution were 100% for obstructive sleep apnea, 90% for hypertension, 66% for joint disease and 62% for type 2 diabetes mellitus.

Conclusion: Bariatric surgery is a safe and effective treatment for EOP with similar results in weight loss and comorbidity resolution than in the age group <60 years without a higher rate of complications. As observed in other studies, at our institution with a multidisciplinary team bariatric surgery is a safe and effective treatment for weight loss and comorbidity improvement in carefully selected EOP. Under these circumstances, access to surgery of this age group should not be limited.

P189-T | Comparative study between gastric bypass and sleeve gastrectomy at the Clínica Universidad de Navarra

C. Tuero*; P. Ahechu*; F. Rotellar*; M.L. Irigoyen†; M. Molina†; R. Moncada‡; M. Landecho§; C. Silva¶; J. Salvador¶; G. Frühbeck¶; V. Valenti*

Department of Surgery, Clinica Universidad de Navarra, Pamplona, Spain; †Assistant Nurse, Clínica Universidad de Navarra, Pamplona, Spain; ‡Department of Anesthesiology, Clinica Universidad de Navarra, Pamplona, Spain; §Department of Internal Medicine, Clinica Universidad de Navarra, Pamplona, Spain; ¶Department of Endocrinology & Nutrition, Clinica Universidad de Navarra, CIBEROBN, IdiSNA, CIBEROBN, IdiSNA, Pamplona, Spain

Background: In the last decades an increase in the application of bariatric surgery has taken place, but clear evidence of which technique is better suited for each patient has not been provided. The aim of this study was to compare our long-term results between the two main bariatric techniques: laparoscopic sleeve gastrectomy (SG) and laparoscopic gastric bypass (GB).

Material and methods: We performed a prospective study with 502 patients who underwent bariatric surgery at our hospital, the Clínica Universidad de Navarra, with a 5-year follow-up with our multidisciplinary team. SG (25·6%) and GB (74·4%) outcomes were compared as regards weight loss, morbidity and comorbidity resolution.

Results: No statistical differences were reported in both groups in surgical complications (7·2% GB vs 5·5% SG). Mortality rate was 0%. The days of hospital admission were three in both groups. BMI mean of the whole sample was 48·3 kg/m^2. Total weight loss at 1 and 5 years was 32·7% and 28·5% for GB and 28·5% and 19·2% for SG. Excess BMI loss at 1 and 5 years for GB was 84% and 70% as compared to 71% and 70%, respectively, for SG. Statistical differences between both surgical techniques were observed in the improvement of comorbidities. The percentages of resolution at 5 years of type 2 diabetes mellitus, hypertension and dyslipidaemia were 75%, 81% and 95% for GB, and 60%, 59% and 67% for SG, respectively.

Conclusion: Bariatric surgery is an effective and safe treatment for morbidly obese patients with a low rate of complications, good weight loss results and a high rate of comorbidity resolution. At our hospital gastric bypass proved to be more effective exhibiting better long-term weight loss results and a higher percentage of comorbidity resolution.

P190-T | The IGFs system in response to an inadequate diet

S. Guerra-Cantera*,†,‡; F. Díaz*,†; P. Ros‡,§; A. Freire-Regatillo*,†,‡; M. Torrecilla-Parra‡; S. Canelles*,†; V. Barrios*,†; C. Oxvig¶; J. Frystyk¶; J. Chowen*,†,**; J. Argente*,†,‡, **

**CIBEROBN, Madrid, Spain; †Hospital Infantil Universitario Niño Jesús, Madrid, Spain; ‡Universidad Autónoma de Madrid, Madrid, Spain; §Hospital Universitario Puerta de Hierro, Majadahonda, Spain; ¶Aarhus University, Aarhus, Denmark; **IMDEA Food Institute, CEI UAM + CSIC, Madrid, Spain*

Background: High fat diet (HFD) increases adiposity and causes harmful effects regardless of weight gain. The insulin-like growth factor (IGF) system is involved in growth and metabolism, as well as neuroprotection mediated in part through astrocytes. Little is known regarding the direct effect of nutrients on the IGF system, especially on the newest members of this family, the pappalysins (PAPP-A) and stanniocalcins (STCs). We aim to determine how dietary signals modulate the peripheral and central IGF systems.

Material and methods: To determine the rapid dietary effect, male and female Wistar rats were given a HFD (60% fat, 5·1 kcal/g), LFD (10% fat, 3·76 kcal/g) or rat chow (Ct, 3·1% fat, 2·9 kcal/g) for 1 week (n = 6). Primary astrocyte cultures were treated 24 h with palmitic acid (PA, 0·25 mM), IGF1 (10 or 50 ng/mL) or both. Gene expression was measured by RT-PCR. Serum hormone levels were measured by ELISA.

Results: HFD increased weight in males ($P < 0·03$), but not females. Energy intake was higher on HFD in males ($P = 0·002$) and females ($P < 0·03$), and LFD in males ($P < 0·03$) and females ($P < 0·03$) compared to Ct.

Males had higher serum levels of free and total IGF1, IGF-binding protein (BP)3, IGFBP5, insulin, leptin and triglycerides than females. Females had higher PAPP-A2 levels compared to males. LFD increased hypothalamic IGF2 mRNA levels in both sexes, being statistically significant in males ($P < 0·02$).

In hypothalamic and hippocampal astrocytes, PA markedly decreased the IGF1 system, except PAPP-A and STC-2, which increased. Exogenous IGF1 did not block the endoplasmic reticulum stress and cytokine production induced by PA.

Conclusions: (1) LFD stimulates hypothalamic IGF-2 expression, possibly due to its high carbohydrate composition.

(2) The IGF system in astrocytes is dramatically modified by PA, which could underlie some of the harmful effects of HFD in the brain.

P191-T | Extracellular features of spikes in the neonatal rat CA3 hippocampal pyramidal cells

M. Mukhtarov; E. Juzekaeva; A. Nasretdinov

Laboratory of Neurobiology, Kazan (Volga Region) Federal University, Kazan, Russian Federation

Extracellular currents generated during action potentials are characterized by various amplitudes and waveforms depending on neuronal type and location of the recording electrode relatively to different neuron compartments. Using simultaneous whole-cell recordings from CA3 pyramidal cells and adjacent extracellular recordings we aimed to identify the extracellular features of spikes in neonatal rat CA3 pyramidal cells along the soma and apical dendrites. Experiments were performed on 500-μm thick horizontal brain slices prepared from postnatal day (P) 4–7 Wistar rats. Whole-cell recordings were performed in current-clamp mode, for extracellular recordings 16-shank silicone probe with 100-μm separation distance between the shanks was used. We found that maximal negative phase of the extracellular spikes was observed in the vicinity of soma, and it coincided with the first derivative of the action potential. This perisomatic negative phase coincided with a positive local field potential at the level of apical dendrite. The negative wave further propagated from soma along the apical dendrite at a velocity of about 0·3 m/s with a progressive decrement in amplitude suggesting dendritic backpropagation of the action potential. While the action potentials contribute to the local field potentials during network driven activities, the established here extracellular features of spikes in neonatal CA3 pyramidal neurons can be useful in analysis of the network driven events.

This work was supported by the subsidy allocated to Kazan Federal University for the state assignment no 6.5408.2017/9.10 in the sphere of scientific activities and performed in the framework of the Program of Competitive Growth of Kazan Federal University.

P100-F | Gender features of the degree of obesity among adolescents in the city and village

E. Valeeva

Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russian Federation

The social importance of the problem of obesity is determined by a decrease in life expectancy, the growth of concomitant diseases and disability. The purpose of this work was the study of anthropometric indices for determining fat mass and assessing their prognostic value in adolescents in urban and rural areas of the Republic of Tatarstan. Methods of body mass index, somatometry, physiometry. 247 adolescents aged 11 to 13 years were under observation. Comparative analysis among girls revealed that the body mass index (BMI) among urban girls was 25–25·8 kg/m^2, in rural—19–21 kg/m^2. In the group of boys, these figures are significantly less. At 11 years of age, there was a body mass deficit of BMI <18·5 kg/m^2 in the boys of the study groups, at 13 years there was a significant increase in BMI in urban boys, which was 26–26·4 kg/m^2. Systolic blood pressure at the age of 11 at the beginning of the school year was 88·17 ± 2·16 mm Hg, and at the end—95·75 ± 2·09 mm Hg, the differences are reliable $P < 0·05$. Between the body weight and systolic blood pressure at the age of 11 years, a reliable correlation coefficient is determined. In the study groups sexual differences in the level of blood pressure and heart rate were revealed at the age of 13, with the heart rate in the group of urban schoolchildren significantly higher. Thus, the frequency of obesity is higher among boys living in urban areas, in rural areas—they correspond to the age norms. Source of financing "This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities". State assignment 19.9777.2017/8.9

P101-F | Histological and immunohistochemical characterization of the inguinal scWAT depot in a model of diet-induced obese rats subjected to SG

I. González-Borja*; M. Martín*; A. Rodríguez[†,‡]; J. Gómez-Ambrosi[†,‡]; B. Ramírez[†,‡]; V. Valentí; R. Moncada[‡,§]; J.A. Cienfuegos[‡,§]; G. Frühbeck[†,‡,¶]; V. Catalán[†,‡]; S. Becerril[†,‡]; M.A. Burrell*

**Department of Histology and Pathology, Universidad de Navarra, IdiSNA, Pamplona, Spain; [†]Metabolic Research Laboratory, Clínica Universidad de Navarra, IdiSNA, Pamplona, Spain; [‡]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain; [§]Department of Surgery, Clínica Universidad de Navarra, IdiSNA, Pamplona, Spain; [¶]Department of Endocrinology and Nutrition, Universidad de Navarra, Pamplona, Spain*

Background: Considering the beneficial effect of sleeve gastrectomy (SG) on the reduction of the weight of subcutaneous white adipose tissue (scWAT), the aim of the present study was to characterize, histologically and immunohistochemically, the inguinal scWAT depot in a model of diet-induced obese rats subjected to SG.

Material and methods: Young male Wistar rats ($n = 27$) were put in a diet-induced obesity (DIO) programme with ad libitum access to a high-fat diet (HFD) during 12 months. After that, rats were ranked by final body weight to identify the obesity-prone (OP) ($n = 13$) and obesity-resistant (OR)

(n = 14). OP and OR rats were submitted to surgical treatments (sham operation, SG and pair-fed to the amount of food eaten by sleeve gastrectomized rats). We characterized the inguinal scWAT depot, using conventional stains and immunohistochemistry with antibodies against uncoupling protein-1 (UCP-1), α-smooth muscle actin, CD68 and caspase-3.

Results: CD68 + macrophages were found organised in crown-like structures (CLS) or in clusters. Unexpectedly, no significant differences were found in the number of macrophages between the study groups. We also found lipofuscin, the pigment product of digestion in macrophages. There were significant differences between the groups of the experiment: OP rats exhibited significantly more lipofuscin than OR (P = 0·01) in the total area of the scWAT section. We did not observe browning in scWAT in any group of rats of the experiment. However, there was a well-developed mammary gland in the inguinal scWAT.

Conclusion: Our findings suggest major differences in the inguinal scWAT of Wistar rats. These differences have to be considered when using male Wistar rats as a model in obesity research.

Financial support: This work was supported by Fondo de Investigación Sanitaria-FEDER (PI16/00221 and PI16/01217). CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn) is an initiative of the Instituto de Salud Carlos III, Spain.

P102-F | Circulating miRNAs as predictive biomarkers of type 2 diabetes mellitus development: from the CORDIOPREV study

A. Camargo*,†,‡,§; R. Jimenz-Lucena*,†,‡,§; Juan.F. Alcalá-Diaz*,†,‡,§; C. Vals-Delgado*,†,‡,§; J. Lopez-Moreno*,†,‡,§; A. Leon-Acuña*,†,‡,§; A.P. Arenas-Larriva*,†,‡,§; J. Delgado-Lista*,†,‡,§; P. Pérez-Martínez*,†,‡,§; O.A. Rangel-Zuñiga*,†,‡,§; J. Lopez-Miranda*,†,‡,§

**CIBER Fisiopatologia de la Obesidad y la Nurtricion (CIBEROBN), Cordoba, Spain; †Lipids and Atherosclerosis Unit, Reina Sofia University Hospital, Cordoba, Spain; ‡Maimonides Institute for Biomedical Research of Cordoba (IMIBIC), Cordoba, Spain; §University of Cordoba, Cordoba, Spain*

Background: Circulating miRNAs have been proposed as type 2 diabetes mellitus (T2DM) biomarkers, and may be a more sensitive way to predict development of the disease than the currently used tools. Our aim was to identify whether circulating miRNAs, added to clinical and biochemical markers, could have better potential predicting T2DM.

Material and methods: The study included 462 patients without disease at baseline from the CORDIOPREV trial. After a median follow-up of 60 months, 107 of the subjects developed the disease. Plasma levels of 24 miRNAs were measured by qRT-PCR. Diabetes risk test (FINDRISC) and other strong biomarkers to predict diabetes such as HbA1c were determined. The potential predictive value of the circulating miRNAs in the development of T2DM was evaluated by OPLS-DA, ROC and Cox regression analysis. The risk of developing T2DM based on baseline miRNAs plasma levels was evaluated for each miRNA by Cox regression analysis and categorizing patients by tertiles (T1-T2-T3).

Results: Plasma levels of 24 miRNAs were measured at baseline by qRT-PCR and other strong biomarkers to predict diabetes were determined. The ROC-analysis identified 9 miRNAs, which added to HbA1c, have a greater predictive value in early diagnosis of type 2 diabetes (AUC = 0·833) than HbA1c alone (AUC = 0·695). The miRNAs and HbA1c based model did not improve when the FINDRISC was included (AUC = 0·829). Cox-regression analyses showed that patients with low miR-103, miR-28-3p, miR-29a, miR-9 and high miR-30a-5p and miR-150 circulating levels have higher risk of disease (HR = 11·68; 95% CI: 3·56–38·34).

Conclusion: Our results suggest that an alteration in the plasma levels of miRNAs mechanistically related with T2DM precede its development and that these features may be used as a predictive biomarker to evaluate the risk of developing T2DM.

P103-F | Alpha cell function interacts with diet to modulate prediabetes and type 2 diabetes

I.R. Ramos*,†,‡,§; R. Jimenez-Lucena*,†,‡,§; J. Lopez-Moreno*,†,‡,§; Juan.F. Alcala-Diaz*,†,‡,§; C. Vals-Delgado*,†,‡,§; Antonio.P. Arenas-Larriva*,†,‡,§; Oriol.A. Rangel-Zuñiga*,†,‡,§; A. Leon-Acuña*,†,‡,§; J. Delgado-Lista*,†,‡,§; P. Perez-Martinez*,†,‡,§; A. Camargo*,†,‡,§; J. Lopez-Miranda*,†,‡,§

**CIBER Fisiopatologia de la Obesidad y la Nutricion (CIBEROBN), Instituto de Salud Carlos III, Cordoba, Spain; †Lipids and Atherosclerosis Unit, Reina Sofia University Hospital, Cordoba, Spain; ‡Maimonides Institute for Biomedical Research of Cordoba (IMIBIC), Cordoba, Spain; §University of Cordoba, Cordoba, Spain*

Background: Alpha- and beta-cells dysfunction is implicated in the development of type 2 diabetes mellitus (T2DM). We aimed to evaluate whether alpha- and beta-cell dysfunction may precede prediabetes (PreDM) and T2DM development. Furthermore, we explored the role of two healthy diets (Mediterranean and low-fat diets) modulating these processes.

Material and methods: We included 462 patients from the CORDIOPREV study without T2DM at baseline, of which 272 were PreDM. During follow-up, 107 patients

developed T2DM (T2DM-incident group), 30 developed PreDM (PreDM-incident group), 86 regressed to normoglycemia (PreDM-regression group) and 29 patients remained without PreDM or T2DM criteria (control group), according to the American Diabetes Association diagnosis criteria. We measured glucose, insulin, glucagon and GLP-1 plasma levels in the OGTT performed at baseline and after 2 years of follow-up. Patients were randomized to consume two healthy diets, a Mediterranean (>35%) and a low-fat (<30%).

Results: At baseline we already observed higher levels of glucagon and glucagon/insulin (G/I) ratio in the T2DM-incident group compared with PreDM-incident and control groups. T2DM Risk Assessment by COX analysis using G/I ratio at 30 minutes after an OGTT was able to assess the T2DM risk with an HR of 2·514. The two dietary models differentially influenced the PreDM regression. Specifically, the consumption of Mediterranean diet was associated with a decrease in G/I ratio ($P = 0{\cdot}034$), whereas the low-fat diet reduced insulin levels ($P = 0{\cdot}002$).

Conclusion: Our results suggest that alpha-cell dysfunction precedes the T2DM development. This process seems to be independent of diet consumed. However the PreDM regression might be differentially modulated by diets.

P104-F | TWEAK/FN14 axis in obesity is regulated by a hypocaloric diet supplemented with EPA and/or α-lipoic acid

X. Escoté; A.E. Huerta; M. Fernadez-Galilea; E. Félix-Soriano; N. Sainz; J.A. Martinez; M.J. Moreno-Aliaga

Centro De Investigación En Nutrición, Universidad De Navarra, Pamplona, Spain

The proinflammatory state associated to obesity is critical to develop some accompanying comorbidities and complications. Tumour necrosis factor weak inducer of apoptosis (TWEAK) is associated with obesity, diabetes and insulin resistance. However, data concerning the potential regulation of the TWEAK/FN14 axis by weight loss and the effects of supplementation with EPA/α-lipoic acid in healthy overweight/obese women is not available. This study is a short-term placebo-controlled trial where women received the allocated treatment: Control, EPA (1·3 g/d), α-lipoic acid (0·3 g/d), and EPA+α-lipoic acid (1·3 g/d; 0·3 g/d) following an energy-restricted diet of 30% less than total energy expenditure. Plasma Tweak levels and TWEAK and FN14 expression in adipose tissue biopsies and PBMC were analyzed. The hypocaloric diet promoted a decrease in TWEAK levels, associated with changes in inflammatory and insulin resistance biomarkers. Moreover, TWEAK expression was unmodified in adipose tissue and PBMC after the intervention, but in PBMC FN14 mRNA presented a decreased expression in the EPA groups. The intervention suggests that in healthy overweight/obese women TWEAK levels decreased in response to a calorie restricted diet, in parallel with a better insulin sensitivity profile. These changes in circulating TWEAK are not accompanied by changes in the PBMC or adipose TWEAK mRNA expression, indicating that other mechanisms are implicated in the regulation of the systemic levels of Tweak.

P105-F | The misclassification regarding the diagnosis of overweight and obesity by using BMI deprives migraineurs of therapeutic strategies

S. Romero*; A. Mínguez-Olaondo†; J.M. Laínez‡; E.A. Martínez-Vila†; G. Frühbeck*; P. Irimia†

**Endocrinology, Clínica Universidad de Navarra, CIBER Fisiopatología De La Obesidad Y Nutrición (CIBERobn), ISCIII, Spain, Pamplona, Spain; †Department of Neurology, Clínica Universidad de Navarra, Pamplona, Spain; ‡Neurology, Hospital Clínico Universitario de Valencia, Valencia, Spain*

Background: Obesity is associated with an increased risk of migraine. Body mass index (BMI) is the diagnostic tool widely used to classify obesity, but this method underestimates its prevalence, defined as an increase in body fat percentage (BF%). We aimed to examine the potential misclassification regarding the diagnosis of overweight and obesity by using BMI as compared with the determination of BF% (Bod Pod®) in migraineurs.

Material and methods: Sixty one patients (18–49 years-old), 46 with episodic migraine and 15 with chronic migraine, underwent BMI and Bod Pod® exams. Patients with known comorbidities such as severe or systemic disease, pregnancy or breastfeeding, major psychiatric disorders, immunosuppression or morbid obesity, according to BMI were excluded from the study. Age, Bod Pod® parameters and anthropometric data were analysed. We performed a descriptive analysis to assess misclassification on the diagnosis of obesity using BMI as compared with BF% and Cohen's Kappa Coefficient Index to evaluate the quality of agreement.

Results: We found that 1 (1·6%) patient was classified as underweight, 43 (70·5%) normal weight, 13 (21·3%) overweight and 4 (6·6%) obese according to BMI. Using BF% 2 (3·3%) patients were classified as underweight, 19 (31·1%) patients as normal weight, 13 (21·3%) as overweight and 27 (44·3%) as obese. Cohen's Kappa Coefficient Index value was 0·210 which is no more than a fair degree of agreement. More than 70% of patients between

38 and 50 years-old were misclassified by BMI. The misclassification according to BMI was observed in normal weight and overweight migraineurs.

Conclusions: Our findings suggest that a relevant number of migraine patients are misclassified according to BMI as compared with BF% because of the fair degree of agreement. This misclassification could deprive migraineurs of therapeutic strategies and raise health cost. Replication of present findings in wider populations are warranted.

P106-F | A score of traditional and novel lifestyles for optimal blood pressure

J. Díaz-Gutiérrez*; L. Ruiz-Estigarribia*; M. Bes-Rastrollo*,†,‡; M. Ruiz-Canela*,†,‡; A. Gea*,†,‡; M.Á. Martínez-González*,†,‡,§

Department Of Preventive Medicine And Public Health, School Of Medicine, University Of Navarra, Pamplona, Spain; †IDISNA Navarra's Health Research Institute, Pamplona, Spain; ‡Biomedical Research Network Center for Pathophysiology of Obesity and Nutrition (CIBEROBN), Carlos III Health Institute, Madrid, Spain; §Department of Nutrition, Harvard TH Chan School of Public Health, Boston, United States

Background: Lifestyle contributes to the risk of hypertension. A healthy-lifestyle score has been associated with lower risk of cardiovascular disease and it might be also related to hypertension. Our objective was to assess the association between a healthy-lifestyle score and the incidence of hypertension.

Methods: The SUN Project is a dynamic, prospective cohort of Spanish university graduates with a retention proportion of 91%. In 14 057 participants initially free of hypertension, we calculated a healthy-lifestyle score based on a 10-point score that we previously reported to be associated with lower risk of clinical cardiovascular events: never smoking, physical activity (>20 METs-h/wk), Mediterranean diet adherence (≥4/8 points), low body mass index ≤22 kg/m^2, moderate alcohol intake (women 0·1–5 g/d, men 0·1–10 g/d), no binge drinking (never >5 drinks/d), reduced exposure to television (<2 hour/d), sleeping a short mid-day nap (≤30 minutes/d), meeting up with friends >1 hour/d and working ≥40 hour/wk. The last four factors showed no association with the risk of hypertension. We used the other 6 factors as exposures to create a 6-point hypertension prevention score and we fitted Cox regression models to adjust for potential confounders.

Results: During a median follow-up of 10·2 years, we identified 1406 incident cases of medically-diagnosed hypertension. The lowest risk of developing hypertension was obtained when participants adhered to a healthy lifestyle pattern by summing up these 6 factors. The highest category (5–6 factors) exhibited a significant 46% relative reduction in the risk of developing hypertension compared to the lowest category (0–1 factors) (multivariable-adjusted hazard ratio = 0·54; 95% CI: 0·42–0·68).

Conclusions: A healthy-lifestyle score including six simple healthy habits was longitudinally and independently associated with a substantially reduced risk of hypertension. This index may be useful for hypertension prevention.

P107-F | Caloric restriction in rats fed a high-fat diet normalizes body weight and adiposity, but not glucose homeostasis

M. Martín*; A. Rodríguez†,‡; J. Gómez-Ambrosi†,‡; B. Ramírez†,‡; S. Becerril†,‡; M. López‡,§; C. Diéguez‡,§; G. Frühbeck†,‡,¶; M.A. Burrell*,‡

Department of Histology and Pathology, Universidad de Navarra, IdiSNA, Pamplona, Spain; †Metabolic Research Laboratory, Clínica Universidad de Navarra, IdiSNA, Pamplona, Spain; ‡CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud, Carlos III, Madrid, Spain; §Department of Physiology Universidad de Santiago de Compostela, Instituto de Investigación Sanitaria (CIMUS), Santiago de Compostela, Spain; ¶Department of Endocrinology and Nutrition, Clínica Universidad de Navarra, IdiSNA, Pamplona, Spain

Background: Energy restriction is a first therapy in the treatment of obesity, but the underlying biological mechanisms have not been fully elucidated. We analysed the effects of restriction of high-fat diet (HFD) on weight loss, glucose homeostasis, circulating gut hormone levels and expression of hypothalamic neuropeptides.

Material and methods: Ten-week-old male Wistar rats (n = 40) were divided into four groups: two fed ad libitum a normal diet (ND) (N group) or a HFD (H group), and two subjected to a 25% caloric restriction of ND (NR group) or HFD (HR group) for 9 weeks. Adiposity index, circulating concentrations of glucose, insulin, leptin, ghrelin, PYY and GLP-1 were measured. Expression of neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART) in the arcuate nucleus of the hypothalamus (ARC) were assessed by in situ hybridization.

Results: A 25% restriction on HFD over 9 weeks entails a 36% weight loss accompanied by normal values in adiposity index and food efficiency ratio (FER). In the HR group normalization of ghrelin and leptin circulating levels as well as hypothalamic expression of NPY, AgRP and POMC was observed. However, the reduction in food consumption did not succeed in improving glucose homeostasis and avoided neither the HFD-induced hyperglycaemia as well as nor normalization of circulating levels of PYY and reduced CART expression.

Conclusions: The 25% restriction on HFD normalized body weight, adiposity and ghrelin and leptin levels. This restriction also entails normal expression of hypothalamic NPY, AgRP and POMC mRNA but did not improve glucose homeostasis.

Financial support: This work was supported by Ministerio de Economía y Competitividad (SAF2015-71026R, to M.L.) and Fondo de Investigación Sanitaria-FEDER (PI16/00221 and PI16/01217). CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn) is an initiative of the Instituto de Salud Carlos III, Spain.

P108-F | Does metabolic syndrome cause hepatic damage? Study of the therapeutic effect of melatonin

A. Díaz-Luis*; Y. Potes*,†; I. Menéndez-Valle*; L. Fernández-Calleja*; B. de Luxán-Delgado‡; Juan.J. Solano†,§; I. Vega-Naredo*,†; A. Coto-Montes*,†; B. Caballero*,†

**Department of Cell Biology and Morphology. University of Oviedo, Oviedo, España; †Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, España; ‡Department for Cancer Stem Cells and Ageing, Barts Cancer Institute, Queen Mary University of London, London, England; §Geriatric Service, Monte Naranco Hospital, Oviedo, España*

Obesity is one of the most important concerns in the current society and has experimented a huge increase during the last decades. The combination of obesity with other types of alterations such as insulin resistance, decrease in HDL levels, hypertension and increased triglycerides, leads to the development of an endocrinopathology know as metabolic syndrome, being the point at which we will focus our study.

To emulate this situation we carried out two experiments, a short experiment (TS) during five weeks and long experiment (TL) during ten weeks. Each one of them was constituted of sixteen individuals of Syrian golden hamster who were fed a high-fructose diet to induce metabolic syndrome. Half of the animals from both experiments were treated with melatonin in order to study the effect of this indolamine on metabolic syndrome derived alterations.

This study showed that melatonin treatment affected antioxidant capacity depending on the type of diet that was administered. Furthermore, an increase in the total antioxidant activity as well as in the activity of catalase was observed in the control animals treated with melatonin.

Obtained data showed that high-fructose diet has not induced metabolic syndrome at hepatic level significantly during short and long treatment. On the other hand, melatonin treatment acts as a direct and indirect antioxidant on animals that were not fed high-fructose diet from both experiments.

Acknowledgements: This work was supported by PI13/02741, PI17/02009 and GRUPIN-071. Y.P. is a FISS predoctoral fellow (FI14/00405).

P109-F | The effect of palmitic acid and DHA supplementation on DNA methylation of inflammatory genes in THP-1 macrophages

J. Alfredo Martínez*,†,‡; M. Samblas*,†; F.I. Milagro*,†,‡

**Department of Nutrition, Food Science and Physiology, University of Navarra, Pamplona, Spain; †Centre for Nutrition Research, University of Navarra, Pamplona, Spain; ‡Biomedical Research Centre in Physiopathology of Obesity and Nutrition, CIBERobn, Madrid, Spain*

Background: Fatty acids may regulate body inflammatory responses. In particular, saturated fatty acids such as palmitic acid induce inflammatory signals in macrophages. However, beneficial effects of polyunsaturated fatty acids belonging to the n-3 family including docosahexaenoic acid (DHA) on inflammation have been reported. In addition, several studies have evidenced the role of fatty acids on DNA methylation, regulating gene expression in inflammatory processes. The aim of this study was to evaluate the effect of the supplementation with palmitic acid or DHA on DNA methylation and the relationships with the expression of inflammatory markers in THP-1 macrophages.

Material and methods: THP-1 cells (human monocytes) were differentiated into macrophages with 25 ng/mL of phorbol 12-myristate 13-acetate (TPA), followed by incubation with 80 μM of palmitic acid or DHA for 24 hour. RNA and DNA was extracted, and cell medium was stored at −80°C. IL18 and TNFα gene expression was measured by qRT-PCR and protein levels by ELISA. The methylation of CpG sites in IL18 and TNFα were analyzed by MassARRAY EpiTYPER.

Results: The incubation of THP-1 macrophages with palmitic acid increased IL18 and TNFα gene expression and protein levels. In addition, the treatment with DHA decreased IL18 and TNFα gene expression and protein levels. However, the supplementation with these fatty acids did not apparently modify the DNA methylation status of these genes in the investigated CpG sites.

Conclusions: This study reveals that the incubation with palmitic acid increases important inflammatory markers in macrophages, whereas DHA decreases the inflammatory response. Apparently, DNA methylation is not directly involved in the fatty acid-mediated regulation of the expression of these genes.

P110-F | Leptin deficiency induces inflammation and endoplasmic reticulum stress in brain from ob/ob mice

A. Rubio-González*; Y. Potes*,†; J.C. Bermejo-Millo*; L. Fernández-Calleja*; I. Menéndez-Valle*; J.J. Solano‡; B. Caballero*,†; A. Coto-Montes*,†; I. Vega-Naredo*,†

*Department of Cell Biology and Morphology, Faculty of Medicine, University of Oviedo, Oviedo, Spain; †Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain; ‡Geriatric Service, Monte Naranco Hospital, Oviedo, Spain

Obesity is a health problem due to overly energy-rich diets and sedentary lifestyle in our modern societies. Obesity promotes multiple cellular processes that attenuate leptin signaling and increase oxidative stress leading to many obesity-related disorders that can reach nervous system. We studied in leptin-deficient mice, what disorders are induced into the brain by obesity and how important antioxidant, as melatonin, can mitigate it.

For that purpose, half of wild-type and leptin-deficient obese mice that were treated with melatonin (500 μg/kg) for 4-weeks and evaluated unfolded protein response (UPR) and subsequent inflammation.

ATF-6α increase was observed in leptin-deficient mice, while IRE1α was maintained and p-eIF2α showed significant decrease. Melatonin treatment was able to reduce this UPR in ob/ob mice until control levels. This UPR observed in ob/ob induced inflammation denoted by significant IL-6 increase and TNF-α decrease. Melatonin induced increase of both cytokines. Thus, obesity induces increase in inflammation and endoplasmic reticulum stress in brain, while melatonin is able to decrease them.

Therefore, melatonin administration might be a good strategy against the deleterious effect of obesity in brain and a potential therapeutic agent against the brain damage induced by leptin-deficient obesity.

Acknowledgements: This work was supported by PI13/02741, PI17/02009 and GRUPIN-071. Y.P. is a FISS predoctoral fellow (FI14/00405).

P111-F | Evolution of cardiometabolic risk parameters after a diet low in FODMAPs

P.Y. Esquíroz*; A.S. Langarica†; N.V. Rovira‡; P.I. Solano‡; M.Á.M. Coscojuela‡; V.S. Moriones‡; R.A. Musgo§; M.B. Ibáñez§; C.C. Ribón§; S.Dl.R. Onandía§; M.H. Bayod§; C.P. de Frías§; C.L. Arregui¶; C.S. Froján‡

*Clinical Nutrition Services, Clínica Universidad De Navarra, Pamplona, Spain; †School of Medicine, Universidad de Navarra, Pamplona, Spain; ‡Department of Endocrinology and Nutrition, Clínica Universidad de Navarra, CIBEROBN, IdiSNA, Pamplona, Spain; §Department of Gastroenterology, Clínica Universidad de Navarra, Pamplona, España; ¶Pharmacy Services, Clínica Universidad de Navarra, Pamplona, España

Background: Cardiovascular disease is the leading cause of mortality in high-income countries. The prevalence of functional digestive disorders, such as irritable bowel syndrome (IBS), reaches 10% of the population. A novel and effective dietetic approach to IBS is the restriction of Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols, known as the low FODMAP diet. There are no studies on the evolution of cardiometabolic risk under this diet, despite the fact that some heart-healthy foods are restricted. The main purpose of this research is to study the evolution of cardiometabolic risk factors after a low FODMAP diet.

Material and methods: We analyzed a retrospective cohort of 50 adult patients with functional digestive disorder who followed a low FODMAP diet.

Patients with a record of dietary consultation and a minimum follow-up of 6–8 weeks, subjective confirmation that they have correctly followed the diet and without medication changes during the study period were included.

Total cholesterol, LDL-c, HDL-c, triglycerides, plasma glucose and weight before (12 months) and after (2–12 months) the beginning of the diet were compared by Student′s t-test. Statistical analysis was carried out by SPSS 22·0.

Results: No significant changes were detected in most cardiometabolic risk factors including total cholesterol, LDL-c, triglycerides, plasma glucose and weight.

A significant increase in HDL-c (6%, $P < 0{\cdot}001$) was observed. A greater increase was observed in subjects with HDL-c levels <60 mg/mL compared to those with > 60 mg/dL (23·2% and 3·4% respectively).

Conclusions: Low FODMAP diet does not seem to negatively modify the cardiometabolic risk. Moreover, a significant increase in HDL-c was observed after the follow-up of a low FODMAP diet.

Low in FODMAP diet could have a role in the treatment of patients with low HDL-c.

More studies are needed to know the health impact of low FODMAP diets.

P112-F | Impact of obesity in oncology patients with advanced stage tumour

P.Y. Esquíroz*; C.L. Arregui†; C.S. Froján‡; M.R. Vázquez§; C.U. Fernández§

*Clinical Nutrition Services, Clínica Universidad De Navarra, Pamplona, Spain; †Pharmacy Services, Clínica Universidad de Navarra, Pamplona, Spain; ‡Department of Endocrinology and Nutrition, Clínica Universidad de Navarra, CIBEROBN, IdiSNA, Pamplona, Spain; §Diets Services, Clínica Universidad de Navarra, Pamplona, Spain

Background: The evaluation carried out on 2016 by the WHO International Agency for Research on Cancer (IARC) for Cancer Prevention based on a systematic review of the published scientific literature concluded that obesity is a risk factor for more cancer sites than previously established. In addition, the review showed that there is sufficient evidence in humans that the absence of excess body fatness in adults reduces the risk of cancers of the gastric cardia, liver, gallbladder, pancreas, ovary, and thyroid.

Material and methods: Inclusion criteria: we selected 88 oncology patients with advanced stage tumour admitted during 2017 with completed Screening Nutritional MUST in this retrospective study. Obesity was defined by a body mass index (BMI) over 30 kg/m^2. BMI is the most common measure to categorize the nutritional status of admitted patients.
Exclusion criteria: patients without current weight, patients with early stage tumour or BMI below 29·9 kg/m^2.
A statistical analysis of the sample was carried out using the Stata program-version 12·0. Due to the non-normal distribution of the studied variables U Mann-Whitney's test was applied to analyse the data.

Results: Including the first alert, more than 74% of the patients had one type of cancer previously related with obesity. More than 44% of the types of cancer were included in the new list of the IARC evaluation. The statistical results showed no difference between the BMI impact on one or the other group compared.

Conclusions: The identification of new obesity-related cancer sites adds to the number of cancer worldwide attributed to obesity. BMI control can decrease the incidence of 12 different types of cancer.

P113-F | Gastric bypass and sleeve gastrectomy are equivalent in their effects on body mass index, fat mass and insulin resistance in diabetic and non diabetic morbid obese subjects

J. Gargallo*; C.M. Perdomo*; M. Llavero*; M. García*; S. Laguna*; J. Escalada*,†; C. Silva*,†; V. Valentí*; J. Gómez-Ambrosi*,†; G. Frühbeck*,†; J. Salvador*,†

University Clinic of Navarra, Pamplona, Spain; †Ciberobn, Spain

Background/Objective: To compare Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) results on BMI, fat mass (Bod-Pod; FM%) and insulin resistance (HOMA-R).

Methods: Retrospective study evaluating 271 patients preoperatively, 1 and 6 months after surgery, age 42 ± 12, 2y (72% women) operated of RYGB (n = 218) or SG (n = 53). Four groups were made according to surgical technique and presence of DM: DM1 m (RYGB n = 47; SG n = 9), noDM1 m (RYGB n = 158; SG n = 41), DM6 m (RYGB n = 27; SG n = 8) and noDM6 m (RYGB n = 104; SG n = 13). Data are shown as mean percentage reduction of each variable studied.

Results: Preoperatively there were significant differences in BMI in DM6 m (RYGB 47·2 ± 8·8 kg/m^2 vs SG 39 ± 5 kg/m^2; $P < 0·05$) and noDM6 m (RYGB 44·5 ± 6·1 kg/m^2 vs SG 40·4 ± 6·7 kg/m^2; $P < 0·05$). No significant differences were observed at baseline BMI in DM1 m (RYGB 47·8 ± 8·9 kg/m^2 vs SG 48·3 ± 9·9 kg/m^2), noDM1 m (RYGB 44·5 ± 6·4 kg/m^2 vs SG 44·3 ± 10·5 kg/m^2), FM% in any group (DM1 m RYGB 51·3 ± 6·8% vs SG 55·9 ± 12·8%; noDM1 m RYGB 52 ± 5·8% vs SG 52·9 ± 19·8%; DM6 m RYGB 51·1 ± 7·6% vs SG 46·8 ± 7·8%; noDM6 m RYGB 52·3 ± 5·8% vs SG 51·5 ± 6·4%) or baseline HOMAR (DM1 m RYGB 8·4 ± 8·1 vs SG 10·4 ± 4·1; noDM1 m RYGB 4·9 ± 3·7 vs SG 5·7 ± 3·7; DM6 m RYGB 8·3 ± 8·5 vs SG 11 ± 7·5; noDM6 m RYGB 5 ± 3·6 vs SG 4·3 ± 2·1). Significant differences were found in BMI percentage reduction at noDM1 m (RYGB −11 ± 3·3% vs SG −12·4 ± 3·47%; $P < 0·05$). Other reductions were no significant: BMI in other groups, FM% (DM1 m RYGB −3·3 ± 6·7% vs SG −7·4 ± 22·5%; noDM1 m RYGB −2·8 ± 5·4% vs SG 1·5 ± 25·8%; DM6 m RYGB −43·7 ± 9·9% vs SG −38·4 ± 13·6%; noDM6m RYGB −23·9 ± 12% vs SG −27·2 ± 7·1%) and HOMAR (DM1 m RYGB −34·5 ± 37·7% vs SG −52·5 ± 23%; noDM1 m RYGB −26·5 ± 57% vs SG −43·1 ± 33·1%; DM6 m RYGB −66·2 ± 27·8% vs SG −56 ± 40·3%; noDM6 m RYGB −39·7 ± 89·8% vs SG −55·7 ± 23·7%).

Conclusions: Significant BMI, FM% and HOMAR percentage reduction in all groups was observed, with no differences between techniques, suggesting both are equivalent improving insulin resistance, body weight and fat mass.

P114-F | Evaluation of the gender differences in the association of visceral fat content and metabolic complications of obesity in a cohort of 743 caucasian subjects

M.F. Landecho*; P. Sunsundegui*; V. Santesteban*; Ó. Beloqui*; I. Colina*; M. García-Unciti*; S. Benito*; A. Arbeola*; G. Frühbeck†; M. Cuervo*

General Health Check-up Unit, Internal Medicine Department, Clínica Universidad de Navarra, Pamplona, Spain; †Department of Endocrinology and Nutrition, Clínica Universidad de Navarra, CIBEROBN, IdiSNA, Pamplona, Spain

Background: Obesity, the XXI-st century pandemia, is defined as an abnormal or excessive fat accumulation that may impair health, being the visceral fat content (VFC) the main cause of its cardio-metabolic consequences. It is mainly diagnosed using the BMI, although it is unable to discriminate neither body fat depot nor its distribution. Besides, body fat distribution as well as metabolic consequences of obesity, are closely gender-related. Our aim was to evaluate the differences in the association between VFC and related metabolic comorbidities in men and women.

Material and methods: We analyzed a population of 743 caucasians, 494 males with a mean age 59 ± 12 years and 249 females, mean age 61 ± 13 years. Anthropometric measurements, blood pressure and biochemistry parameters were collected, as well as medical history of each individual. VFC was measured by Tanita AB-140 (ViScan, Tanita Corp, Tokyo, Japan), an abdominal bioimpedance device, that has been previously validated. Obesity related comorbidities were defined using the metabolic syndrome (MetS) criteria.

Results: The mean VFC observed in subjects with MetS was significantly higher in men (20·0 ± 5..0 u.a.) compared to women (12·6 ± 3·4 u.a.) ($P < 0·001$), being the cut-off points of VFC for the association of MetS in our study population 17·3 u.a. for men and 10·8 u.a. for women.

Conclusions: In our population, the metabolic consequences of VFC differs significantly between men and women, being the latter protected against VFC accumulation but also more susceptible for MetS with smaller increases in VFC.

P115-F | SWATH-MS proteomic analysis of subcutaneous adipose tissue from obese patients in response to bariatric surgery-induced weight loss

R. Guzmán-Ruiz*,†; J. Sanchez-Ceinos*,†; B.C. Townsend*; C.T. Alcazar*; J. López-Alcalá*,†; I. Ortea‡; D.A. Cano§; J.L. Pereira-Cunill§; P.P. García-Luna§; M.M. Malagón*,†

**Department Cell Biology, Physiology, and Immunology, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC)/University of Córdoba (UCO)/Reina Sofia University Hospital (HURS), Córdoba, Spain; †CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), ISCIII, Córdoba, Spain; ‡Proteomics Unit, IMIBIC, Córdoba, Spain; §Unidad de Gestión de Endocrinología y Nutrición, Instituto de Biomedicina de Sevilla (IBiS), Consejo Superior de Investigaciones Científicas, Universidad de Sevilla. Hospital Universitario Virgen del Rocío, Sevilla, Spain*

Background: Bariatric surgery (BS) represents an effective strategy to reduce weight, improve metabolic profile and lower cardiovascular risk factors in morbidly obese patients. Besides decreasing fat mass, BS induces a significant molecular and functional reorganization of the adipose tissue that contributes to its beneficial effects. However, the mechanisms driving these changes have not yet been fully elucidated. Moreover, it is currently accepted that the safety, efficacy and cost-efficiency of BS may vary in relation to the anthropometric and metabolic status (such as BMI or T2D) of obese patients. Thus, novel studies are necessary in order to define the molecular basis of the beneficial effects of BS.

Material and methods: A multi-comparative proteomic analysis using a novel SWATH-MS proteomic approach was employed to analyse the response of subcutaneous adipose tissue to surgery-induced weight loss at 1 year after BS. Bioinformatic analyses (Panther, iPathwayGuide, IPA) were used to identify biological processes of interest. Patients were stratified according to duration of obesity (<15 and >30 years; short- and long-term obesity groups, respectively).

Results: A total of 930 proteins were identified in the human adipose tissue. Quantification by SWATH analysis enabled the identification of 156 proteins that exhibit statistically significant changes after BS, including 93 and 11 proteins that were specifically detected in short-term and long-term obese patients, respectively. Bioinformatic analysis identified fatty acid beta-oxidation and tricarboxylic acid cycle as the main biological processes modified by BS. Notably, these processes were significantly reduced after BS in long-term obesity patients. Other factors, such as the adipogenic regulatory factor (ADIRF) increased independently of obesity evolution.

Conclusion: The beneficial effects of BS on the molecular signature of the obese adipose tissue are largely dependent on the duration of obesity, which is also evident at the systemic level.

Funding: MINECO/FEDER (BUF2016-76711-R; BFU2015-70454-REDT); JJAA/FEDER (PI-0159-2016); ADIPOSEQ, CIBERobn (ISCIII).

P116-F | Kininogen pathway is involved in fat tissue plasticity

M. Peyrou; A. Gavaldà-Navarro; R. Cereijo; M. Giralt; F. Villarroya

Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, Spain

Background: Brown adipose tissue (BAT) plays a beneficial role on metabolism through the induction of thermogenesis, while its inactivation is related to metabolism unbalance and favors obesity and type II diabetes. BAT

might also have an endocrine function that could induce white adipose tissue (WAT) browning and affect other tissues leading to an improvement in the metabolic profile. The secreted products of the Kininogen (Kng) gene play a role in blood coagulation/pressure, pain and inflammation, but the role of these proteins in metabolism is poorly known. We observed that Kng is expressed by BAT and increased in case of BAT activation by cold exposure. Here, we aimed at unraveling the role of Kng in BAT activation and its potential role as BAT endocrine factor.

Material and methods: We performed in vivo experiments using Kng receptors knock-out (KO) mice exposed to cold or thermoneutrality, and measured various parameters using metabolic cages. We also cultured in vitro primary brown/white adipocytes and exposed them to Kng-derived products or β-adrenergic pathway inducers.

Results: When exposed to changes of temperature, mice deficient for Kng receptors showed an impaired brown- and beige-vs-white tissue remodeling and a lack of physiological adaptation. Indeed, KO mice under cold exposure consumed less oxygen and energy and had a reduced food intake compared to controls. They showed a dramatic impairment of induction of browning in WAT, while conversely, WAT lost the capacity of further "whitening" in response to thermoneutrality. Interestingly, Kng-derived proteins had no effect on brown and white adipocytes, indicating that the effect of Kng in mice is not cell-autonomous. However, markers of browning were increased in the KO, confirming the implication of Kng in browning.

Conclusions: All together, these data suggests that Kng is required for the plasticity of adipose tissues occurring in response to challenges in energy balance.

P117-F | Cold exposure affects hypothalamic expression of molecular satiety and leptin signalling-related genes in an age dependent manner

B. Reynés[*,†,‡]; M.K. Hazebroek[§]; E. García-Ruiz[*,†]; J. Keijer[§]; P. Oliver[*,†,‡]; A. Palou[*,†,‡]

[*]Laboratory of Molecular Biology, Nutrition and Biotechnology (LBNB), Universitat de les Illes Balears, Palma, Spain; [†]CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn), Palma, Spain; [‡]Institut d'Investigació Sanitària Illes Balears (IdISBa), Palma, Spain; [§]Human and Animal Physiology, Wageningen University, Wageningen, The Netherlands

Background: Cold exposure produces severe hypoleptinemia, which is linked to compensatory hyperphagia to counteract fat loss related to lipid mobilization and thermogenic activation. However, cold exposure effects on the hypothalamic leptin signalling cascade, and on food intake regulation are not clear and may be affected by age.

Material and methods: We analysed molecular satiety and leptin signalling gene expression by qRT-PCR in different aged rats (1 to 6 months), validated these responses in ferrets, and examined the validity of peripheral blood mononuclear cells (PBMC) as surrogate tissue. Cold stimulus consisted in 1 week at 4°C.

Results: Leptin levels in rats decrease after cold exposure regardless of age and are negatively correlated with food intake. This hypoleptinemia in cold-exposed animals could be responsible for an increased orexigenic/anorexigenic peptide gene expression ratio in the hypothalamus, which is mainly due to decreased anorexigenic gene expression (Cart and Pomc) in young rats (1 month-old), and due to increased orexigenic genes (Agrp and Npy) in ferrets, an animal model closer to humans. Despite the key role of leptin in neuropeptide regulation, expression of leptin signalling-related genes was affected by cold exposure only at the age of 4 months, indicating that some specific factors could affect leptin sensitivity at this particular age in rat hypothalamus. Finally, PBMC were able to reflect some of the characteristic hypothalamic gene expression features of cold exposure observed in our study, both in rodents and ferrets.

Conclusions: In spite that decreased leptin levels after cold acclimation seem to have a critical role in cold-induced hyperphagia, the effect of cold exposure on hypothalamic expression of genes involved in satiation and leptin signalling are age dependent. Moreover, PBMC could be considered as a potential surrogate tissue to perform further studies on the regulation of hypothalamic leptin signalling in response to cold exposure.

P118-F | Circulating endothelial cells and endothelial progenitor cells as possible novel biomarkers for cardiovascular risk assessment: PREDIMED-PLUS Study

R. Casas[*,†]; A.M. Ruiz-León[*]; S. Castro-Barquero[*]; M. Domenech[*]; M. Fitó[†,‡]; J. Salas-Salvadó[†,§]; R. Estruch[*,†]

[*]Department of Internal Medicine, Hospital Clínic, Institut d'Investigació Biomèdica August Pi i Sunyer, Universitat de Barcelona, Barcelona, Spain; [†]CIBER de Fisiopatologia de la Obesidad y la Nutrición (CIBEROBN 0603), Instituto de Salud Carlos III, Madrid, España; [‡]Cardiovascular Risk and Nutrition and REGICOR research group, Hospital del Mar Medical Research Institute (IMIM), Barcelona, España; [§]Human Nutrition Department, University Rovira i Virgili, Reus, España

Background: Circulating endothelial cells (CEC) and endothelial progenitors cells (EPC) are potential biomarkers of endothelial damage in cardiovascular disease (CVD).

The aim of this study was to evaluate the effect of an intensive weight-loss-oriented lifestyle intervention program based on an energy-restricted Mediterranean diet (erMD) plus physical exercise for 1 year on CEC and EPC expression.

Material and methods: 100 older individuals with overweight/obesity and the metabolic syndrome included in the PREDIMED-PLUS trial were randomly assigned to 2 intervention groups: a traditional MD as control group (CG) or an erMD plus intensive lifestyle intervention group (ILI). Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density-gradient centrifugation and, after 1 h incubation, the expression of CEC and EPC were assessed in a FACSCalibur Flow Cytometer. EPCs were identified with KDR-PE, CD34-FITC, CD133-APC and 7-aminoactinomycin D, and CECs with CD34-FITC, CD146-APC, CD45-PE and 7-aminoactinomycin D.

Results: At 1 year, compared to the CG, the ILI group showed more weight loss (−2·05 kg) and reduction in waist circumference (−1·65 cm), increased HDL-cholesterol (2·73 mg/dL), lower triglycerides (−17·4 mg/dL), fasting glucose (−5·8 mg/dL) and HbA1c (−2%), and improved fitness ($P < 0·05$; all). Concomitantly, there was a significant 47% increase of EPCs expression and 57% decrease of CECs in the ILI group ($P < 0·01$).

Conclusion: Nutritional and behavioral intervention appears to modulate endothelial cell expression to further the cardioprotective effect of the MD. CECs and EPcs might be used as complementary biomarkers to provide information on endothelial damage and chronic low-grade inflammation in the atherosclerosis process.

P119-F | Lipid fingerprint of the adipose tissue in obesity and insulin resistance

A. Fernández-Vega*,†; E. Chicano-Gálvez‡; B.M. Prentice††, ‡‡; F. Priego-Capote§; M.A.L. Bascón§; M. Calderón-Santiago§; M. Tena-Sempere*,†; J. López-Miranda†,¶; R. Guzmán-Ruiz*,†; R.M. Caprioli††, ‡‡; M.M. Malagón*,†

**Department Cell Biology, Physiology, and Immunology, IMIBIC/University of Cordoba (UCO)/Reina Sofia University Hospital (HURS), Cordoba, Spain; †CIBER Physiopathology of Obesity and Nutrition (CIBERobn), ISCIII, Spain; ‡Proteomics Unit, IMIBIC/UCO/HURS, Cordoba, Spain; §Department of Analytical Chemistry, IMIBIC/UCO/HURS, Cordoba, Spain; ¶UGC General and Digestive Surgery, IMIBIC/HURS, Cordoba, Spain; ††Mass Spectrometry Research Center, Vanderbilt University, 465 21st Ave S #9160, Nashville, USA; ‡‡Department of Biochemistry, Vanderbilt University, 607 Light Hall, Nashville, USA*

Background: Lipids are essential components of the cells that play important roles in cell signaling, as components of cell membranes, and as fuel molecules. In adipocytes, lipids are used for energy storage, mainly as triacylglycerols, in lipid droplets (LDs). Sphingolipids such as sphingomyelins or ceramides, and ether-phospholipids have recently emerged as key players and potential biomarkers in metabolic disorders, though their biogenesis and turnover are still poorly understood. Likewise, little is known about the specific lipid families that are altered in obesity, in part due to the lack of powerful analytical tools to deal with the complexity of the adipose tissue lipidome.

Material and methods: Herein, we developed a MALDI imaging strategy to map the composition and spatial distribution of lipids in histological sections of adipose tissue from obese subjects with different degrees of insulin sensitivity. Expression levels of the enzymes involved in the biosynthesis of sphingolipids and ether-phospholipids were evaluated during adipogenesis as well as upon exposure of 3T3-L1 adipocytes to hyperglycemia/hyperinsulinemia levels or inflammation environment.

Results: MALDI IMS analysis of adipose tissue sections discriminated more than 7500 features and the PLS-DA analysis of these data enabled the discrimination of diabetic vs non-diabetic patients. Expression studies in adipocytes suggest compartment- and time-dependent activation of the ceramide/sphingomyelin biosynthesis pathways during adipogenesis, while ether-phospholipid synthesis in peroxisomes and endoplasmic reticulum appear at late stages of adipocyte differentiation. The expression of sphingolipid and ether-phospholipid biosynthetic enzymes were greatly impaired upon exposure to TNF-α and, to a lesser extent, by HGHI treatment.

Conclusion: In all, our results suggest that insulin resistance in obesity is associated with the dysregulation of the lipid composition of adipocyte membranes and LDs and strongly support the contribution of hyperglycemia/hyperinsulinemia and inflammatory environment to the dysregulation of both sphingolipids and ether-phospholipids.

Funding: MINECO/FEDER (BFU2013-44229-R; BFU 2015-70454-REDT; BUF2016-76711-R); JJAA/FEDER(PI-0200/2013; PI-0159-2016); FIS(PIE14_00005), CIBERobn (ISCIII).

P120-F | Moringa oleífera, a sustainable alternative: a review on their nutritional and medicinal potential

J. Álvarez-Pérez*,†; A. Sánchez-Villegas*,†; J. Pérez-Cabrera*; F. Díaz-Collado*,†; R.B. García-Guerra*; L. Serra-Majem*,†

**Instituto Universitario de Investigaciones Biomédicas y Sanitarias de la Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain; †Centro de Investigación Biomédica en Red, CIBEROBN, Madrid, Spain*

Background: Moringa oleífera (MO) is a plant with high nutritional and medicinal value, which could prevent and treat human diseases. Native from India, it is now widely distributed throughout tropical and subtropical world regions. Several experimental studies indicate that its leaves have high nutritional components such as vitamins, minerals and amino acids. This narrative review focuses on its possible nutritional and therapeutical potential as a sustainable alternative.

Material and methods: This search was conducted using "moringa oleifera" as a key word within the article title. Other varieties of moringa were excluded.

Results: Several in vitro experiments and animal studies show the potential benefits of MO for human health. Its leaves, pods and seeds contain a large variety of essential phytochemicals (tannins, sterols, saponins, terpenoids, phenolics and flavonoids). The dried MO leaves have a high nutritional value, contributing by 100 g of product: 329 Kcal; protein 29·4 g; fat: 5·2 g; carbohydrates: 41·2 g; fiber: 12·5 g; and vitamins B1: 2·02 mg; B2: 21·3 mg, vitamin C: 15·8 mg; and Vit E; 10·8 mg; calcium: 2185 mg; magnesium; 448 mg; potassium 1236 mg; iron: 25·6 mg. The MO multiple biological activities (antiproliferation, hepatoprotective, anti-inflammatory, antinociceptive, antiatherosclerotic, oxidative DNA damage protective, antiperoxidative, cardioprotective and antimicrobial) are attributed to the presence of functional bioactive compounds (phenolic acids, flavonoids, alkaloids, phytosterols, natural sugars, organic acids).

Conclusions: This review provides an overview on the nutritional values and medicinal properties for commercial and pharmacological use of MO. However, to date the number of clinical trials in humans is scarce. Thus, the level of evidence is low. Further clinical studies are needed to confirm or refute the pharmacological and beneficial effects of MO. Moreover, its safety on human health has to be assessed to ensure its adequacy as a therapeutical tool for chronic or long-term diseases treatment.

P121-F | Maternal polyunsaturated fatty acids dietary supplementation improves obstetrical outcome

B. Virgolici*; M. Mohora*; C. Mehedintu†; C. Berceanu‡; E. Bratila§

** "Carol Davila" University of Medicine and Pharmacy, Department Biochemistry, Bucharest, Romania; †Carol Davila" University of Medicine and Pharmacy, Department of Obstetrics and Gynecology, "Nicolae Malaxa" Clinical Emergency Hospital, Bucharest, Romania; ‡Craiova University of Medicine and Pharmacy, Department of Obstetrics and Gynecology, Craiova County Hospital, Craiova, Romania; § "Carol Davila" University of Medicine And Pharmacy, "prof. dr. Panait Sarbu" Clinical Obstetrica Gynecology Hospital, Bucharest, Romania*

Background: Gestational obesity has high prevalence in the modern era, causing adverse obstetrical outcome. We aim to highlight the benefits of dietary supplementation using polyunsaturated fatty acids on obstetrical prognosis in an experimental model.

Materials and methods: We studied the consequences of gestational obesity fetal prognosis on an animal model using 20 obese female Wistar rats. Obesity was caused by high-fat diet administered by gavage in the rats, after which they were bead. The pregnant rats were separated during gestation into a group receiving polyunsaturated fatty acids (PUFA) and another one that continued the fat diet throughout pregnancy.

Results: Obese Wistar rats were followed throughout gestation and sacrificed at term with their pups. We analyzed the secretion of adipokines from maternal blood (leptin and adiponectin), lipid peroxidation levels by malonyl-dialdehyde (MDA) and antioxidation level by glutathione (GSH) from placental, fetal liver and pancreas homogenates and maternal blood. Decreased levels of adiponectin and increased of leptin concurred with tissue lipid peroxidation measured by elevated MDA and low levels of GSH. Organs histology showed dysplastic epithelial and cells, accumulation of inflammatory cells and congested vessels with thrombosis and glycogen deposits in the fat group. The descendants of the obese mothers throughout were studied and found that they weighed significantly less at birth and were more susceptible to metabolic and infectious diseases compared to the ones from the group that received PUFA supplementation. These pups were more prone to accelerate aging and chronic diseases.

Conclusions: Our study showed a beneficial effect of PUFA supplementation during gestation in obese Wistar rats. The improved obstetrical and fetal outcome in these rats was confirmed through biochemical and histopathological alterations of the intrauterine environment proven by placental dysfunction and the reduced lifespan of the descendants. Considering that, we recommend PUFA supplementation in every pregnancy complicated by obesity.

P122-F | Micronutrient intake adequacy and mortality risk in the PREDIMED study

I. Falcón-García*; A. Ortiz-Andrellucchi*,†; J. Álvarez-Pérez*,†; R. Estruch†; M.A. Martínez-González†; D. Corella†; J. Salas-Salvadó†; M. Fito†; H. Schroder†; E. Toledo†; R.M. Lamuela-Raventós†; E. Ros†; F. Arós†; M. Fiol†; J. Lapetra†; M.A. Muñoz†; E. Gómez-Gracia†; J. Tur†; X. Pintó†; V. Ruiz-Gutiérrez†; M.P. Portillo-Baquedano†; L. Serra-Majem*,†; PREDIMED Study Investigators

**Research Institute of Biomedical and Health Sciences Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Canary Islands, Spain; †Ciber Obn Physiopathology of Obesity and Nutrition, Institute of Health "Carlos III", Madrid, Spain*

Background: Limited prospective studies have examined the association between micronutrient intake adequacy and mortality in general population, previous studies have evaluated such associations taking into consideration participants with specific pathologies or critical conditions. We aimed to prospectively assess the association between micronutrient intake adequacy and all-cause, cardiovascular and cancer-related mortality.

Material and methods: The PREDIMED trial is a randomized, controlled trial conducted in Spain from 2003 to 2011 with 7447 participants at high risk for CVD, aged 55 to 80 years. In a post hoc analysis, we assessed the micronutrient intake adequacy for vitamins B1, B2, B6, B12, C, D, E, folic acid, potassium, iron, magnesium, phosphorus and calcium at baseline. Inadequate intake for each nutrient was defined when the intake of the nutrient was below the estimated average requirements (EAR) if available or below the 50% of the adequate intake level for potassium (EAR was not available). We compared participants with inadequate intake for 2, 3 and ≥4 nutrients vs those with one nutrient or none. Main outcomes were all-cause, cancer and cardiovascular mortality and multivariable-adjusted Cox regression models were fitted to estimate hazard ratios (HR, 95% CI).

Results: A total of 402 deaths were recorded after a median follow-up of 4·8 years. Multivariable-adjusted models revealed no statistically significant difference between micronutrient inadequate intakes and all-cause mortality (≥4 micronutrients inadequacy vs 0–1 micronutrients inadequacy ref HR 1·44; 95% CI 0·99–2·11) neither for cancer and cardiovascular mortality.

Conclusions: No statistically significant association was found between micronutrient intake adequacy and all-cause, cancer, and cardiovascular mortality in elderly subjects at high cardiovascular risk.

P123-F | Association between dairy foods consumption and total and cardiovascular mortality in the PREDIMED study

A. Ortiz-Andrellucchi*,†; A. Sánchez-Villegas*,†; P. Henríquez-Sánchez*,†; R. Estruch†; M.A. Martínez-González†; D. Corella†; J. Salas-Salvadó†; M. Fito†; H. Schroder†; E. Toledo†; R.M. Lamuela-Raventós†; E. Ros†; F. Arós†; M. Fiol†; J. Lapetra†; M.A. Muñoz†; E. Gómez-Gracia†; J. Tur†; X. Pintó†; V. Ruiz-Gutiérrez†; M.P. Portillo-Baquedano†; L. Serra-Majem*,†; PREDIMED Study Investigators

**Research Institute of Biomedical and Health Sciences, Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas. Canary Islands, Spain; †Ciber Obn Physiopathology of Obesity and Nutrition, Institute of Health "Carlos III", Madrid, Spain*

Background: According to several studies, the dairy products, one of the most consumed foods and rich in many nutrients, does not present a conclusive relationship with cardiovascular disease. This study aims to examine the association of dairy foods consumption with all-cause and cardiovascular mortality in a population at high risk for cardiovascular disease included in the PREDIMED study.

Material and methods: This study corresponds to a prospective and observational cohort study carried out in the framework of the clinical trial PREDIMED. A total of 7447 participants aged between 55 and 80 for men and 60 and 80 for women with cardiovascular risk factors, but without cardiovascular disease at the beginning of the study. The data were obtained from questionnaires and/or interviews during the study follow-up period. Four different Cox regression models were used to assess the risks of mortality as a function of the quartiles of intake of dairy products, estimating reasons of risk (HR, 95% CI).

Results: The total number of deaths during the follow-up period was 292, of which 72 were due to cardiovascular causes. Total consumption of dairy products was not related to all-cause and cardiovascular mortality. Consumption of full-fat dairy products, comparing the highest and the lowest intakes, was associated with an increased risk of 67% of all-cause mortality (HR: 1·67, 95% CI: 1·18–2·37) and a 137% of cardiovascular mortality (HR: 2·37, 95% CI: 1·23–4·57). Intake of low-fat dairy products, comparing the highest with the lowest quartile of intake, was associated with a risk reduction of 37% of all-cause mortality (HR: 0·63, 95% CI: 0·45–0·88) and a 59% of cardiovascular mortality (HR: 0·41; 95% CI: 0·21–0·80).

Conclusions: Intake of full-fat dairy products was associated with an increase in all-cause and CVD mortality, while the low-fat dairy consumption was related to a protective association.

P124-F | The A-Body-Shape-Index and Type 2 diabetes are mutually independent predictors of cardiovascular events risk in angiographied coronary patients

A. Vonbank*,†,‡; C.H. Saely*,†,‡,§; C. Lins¶; D. Zanolin*; A. Leiherer*,‡; A. Mader*,†,‡; B. Larcher*,†,‡; A. Muendlein*,‡; H. Drexel*,‡,§,**

*Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; ‡Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; §Division of Angiology, Swiss Cardiovascular Center, Berne, Switzerland; ¶Kantonsspital St. Gallen, Switzerland; **Drexel University College of Medicine, Philadelphia, United States

Background: The A-Body-Shape-Index (ABSI) is calculated based on waist circumference, height and BMI and provides a measure of visceral adiposity. In the general population, this index has been associated with premature mortality. Its power to predict cardiovascular events in high-risk patients is not known and is addressed in the present study.

Material and methods: We prospectively investigated a large series of 1618 patients undergoing coronary angiography for the evaluation of established or suspected coronary artery disease over 6·4 ± 3·2 years.

Results: At baseline, ABSI scores were significantly higher in patients with T2DM (n = 464) than in non-diabetic subjects (14·1 ± 1·3 vs 13·9 ± 1·3; P = 0·003). During follow-up, a total of 494 cardiovascular events were recorded. Cardiovascular event risk was significantly higher in patients with T2DM than in non-diabetic subjects (39·1% vs 27·8%; P < 0·001), and in univariate analysis the ABSI significantly predicted cardiovascular events (HR 1·15 [1·06–1·25]; P = 0·001). In multivariate analyses, both T2DM and ABSI proved independently predictive of cardiovascular events, with standardized adjusted HRs of 1·49 [1·23–1·81]; P < 0·001 and 1·13 [1·04–1·22]; P = 0·004, respectively.

Conclusion: We conclude that ABSI and T2DM are mutually independent predictors of cardiovascular events in angiographied coronary patients.

P125-F | Visceral adiposity is a significantly stronger predictor of diabetes incidence in men than in women

A. Mader*,†,‡

*Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria; †Private University of the Principality of Liechtenstein, Triesen, Liechtenstein; ‡Department of Medicine I, Academic Teaching Hospital Feldkirch, Feldkirch, Austria

Background: Visceral adiposity is a paramount risk factor for the development of type 2 diabetes. Whether it equally increases diabetes risk in men and in women is not known and is addressed in the present study.

Material and methods: We prospectively recorded diabetes incidence in a large high-risk cohort of 1142 nondiabetic patients, including 755 men and 387 women who were undergoing coronary angiography for the evaluation of established or suspected coronary artery disease. Visceral adiposity was measured with the validated visceral adiposity index using waist circumference, serum triglycerides, age and gender to diagnose this metabolic abnormality; diabetes was diagnosed according to ADA criteria.

Results: At baseline, visceral adiposity did not differ significantly between men and women (P = 0·247). Prospectively, 133 (10·4%) of our patients newly developed diabetes during a follow-up period of 3·7 ± 0·9 years. Visceral adiposity significantly predicted the incidence of diabetes in men but not in women both univariately (ORs 1·71 [1·40–2·10]; P < 0·001 and 1·09 [0·81–1·49]; P = 0·565, respectively) and after multivariate adjustment including fasting plasma glucose (ORs 1·56 [1·24–1·97]; P < 0·001 and 1·05 [0·75–1·46]; P = 0·790, respectively). Interaction terms visceral adiposity x gender were significant both for univariate and for multivariate analyses (P < 0·001 and P = 0·017, respectively), indicating that visceral adiposity was a significantly stronger predictor of diabetes among men than among women.

Conclusion: We conclude that in angiographied coronary patients visceral adiposity is a significantly stronger predictor of diabetes incidence in men than in women.Miscellaneous Medical Topics

P126-F | Tension-type headache with myogenic component in adolescents treated by non-pharmacological method

O. Esin*; R. Esin*,†; R. Shamsutdinova*; E. Gorobets*; R. Gamirova*,†

*Kazan (Volga region) Federal University, Kazan, Russian Federation; †Kazan State Medical Academy, Kazan, Russian Federation

Background: It is urgent to reveal the effectiveness of elastic kinesio taping for the treatment of tension headaches (TH) associated with pericranial muscular tension in children because it has not been studied enough.

Material and methods: Diagnosis was conducted according to the International Classification of Headache Disorders, 3rd edition (beta-version). We examined 85 children with frequent episodic and chronic TH with trigger zones in the pericranial muscles, who kept a headache diary for 30 days prior to therapy. The treatment method was

flexible kinesio taping every 5 days for 30 days. Kinesio tapes provide constant support of muscles and tendons, reducing pain and inflammation. The fundamental principle of the method is the modeling of the muscular-fascial segment with the involvement of the skin, subcutaneous tissue, fascial formations, muscles and ligaments.

Results: The average age of the patients was 14.2 ± 2.4 years. The average index of headache intensity according to the Visual Analogue Scale was 63.2 ± 0.9, the average number of days with a headache was 19.3 ± 4.6 days. With the use of muscle-fascial kinesio taping in 30 days, the following results were obtained: the average index of the intensity of the headache by the visual analogue scale was 3.1 ± 0.4, and the average number of days with a headache 2.4 ± 1.1 days ($P < 0.05$). After 30 days, patients were prescribed physical exercise therapy to eliminate muscle imbalance.

Conclusions: Restoring normal muscle work and eliminating myogenic trigger zones is effectively achieved by kinesio taping. An applied tape can both slow down the muscle and facilitate its function. The method is effective in eliminating the myogenic component of TH in children, it is safe, has no contraindications, except for an extremely rare hypersensitivity to the components of the tape.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

P127-F | Exploring semantic analysis of biomedical texts for identification of drugs with similar therapeutic effects

R. Nugmanov*; E. Tutubalina*; Z. Miftahutdinov*; T. Madzhidov*; S. Nikolenko†; I. Alimova*; A. Tropsha*,‡

Kazan Federal University, Kazan, Russian Federation; †Department of V. A. Steklov Institute of Mathematics, Russian Academy of Sciences, St. Petersburg, Russian Federation; ‡University of North Carolina, Chapel Hill, USA

Background: Analysing of textual information for the searching of new therapeutic profiles for existing drugs is a promising field both in academic research and industry. A classic source of such information is scientific works on medicine and biomedicine. However, social networks, discussion boards, and internet forums are widely used by patients to discuss their experience in the use, including off-label use, of various drugs. Such resources could be used as a promising source of biomedical information. In the current work we suggest that textual analysis methods could be used to recover therapeutic effects of the drugs.

Material and methods: A collection of user reviews containing more than 2.5 mln texts was collected. A word2vec model of continuous bag-of-words type was then trained on the corpus in such a way that each word in the collection was assigned with its distributed vector representation.

Results: Similar word vectors were shown to correspond to either drugs with the same active compound or to drugs with close therapeutic effects. At the same time only poor correlation of structural similarity with word embedding similarity was observed. Word embedding fulfill arithmetical relationships that describe semantic and biological similarity: addition of two vectors corresponding to drugs A and B will give a new vector that is similar to the respective vector of a drug that has the properties of both A and B.

Conclusions: Vector word representations better reflects the biological activity than structural descriptors and obey very interesting arithmetic property. This property of word embedding was shown to be readily utilized in drug reprofiling and searching for drugs with required combination of effects.

Acknowledgment: Supported by Russian Government Program of Competitive Growth of KFU, funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7).

P128-F | Detecting adverse drug reactions from user reviews with machine learning

R. Nugmanov; I. Alimova; E. Tutubalina

Kazan Federal University, Kazan, Russian Federation

Background: The automatic extraction of adverse drug reactions from social media becomes one of the actual tools for pharmacovigilance since users publish valuable information about various aspects of their lives, including health care. In this work, we focus on identification of adverse drug reactions from user reviews in English and Russian languages and formulate this problem as a binary classification task.

Material and methods: We applied Support Vector Machine (SVM) and Logistic Regression models with a set of features: a bag of words (bow), part of speech (pos), pointwise mutual information (pmi), sentiment (sent), word embedding (emb), semantic types from Unified Medical Language System (umls), Brown Clusters (cls), precence in drug and adr lexicon (drug_adr), emoticons (emot) for classification. We used vector representation trained on social media posts as word embedding feature for English reviews and ruscorpora vector representation from RusVectores resource for Russian reviews classification. We conducted experiments on CADEC (https://data.csiro.au) and Twitter corpora (http://diego.asu.edu/) for English language and a

dataset of patient reviews about drugs collected from the Otzovik forum for Russian. The CADEC corpus contains 6320 entities, 5770 of them marked as "ADR", the Twitter corpus consists of 732 "ADR" and 6820 of "non-ADR" tweets and for Russian language dataset the statistic is 5748 sentences, 279 of them contain information about "ADR".

Results: We conducted 9 experiments with different sets of features for CADEC and Twitter corpuses and 13 experiments for Russian dataset in order to get the best results of F-measure. On the Twitter corpus, Logistic Regression achieved the macro F-measure of 73.7%. The SVM model obtained the macro F-measure 80.3% for CADEC corpus and 68% for Russian corpus. The results of the experiments with the best sets of features for datasets are presented here:
Features:

bow, pos, sent, cls, umls: Precision (84.4%)/Recall (77.3%)/F-measure (80.3%)
bow, pos, sent, cls, umls, pmi, emb, drug_adr, emot: Precision (72.9%)/Recall (74.6%)/F-measure (73.7%)
bow, w2v, pmi: Precision (65.1%)/Recall (72.9%)/F-measure (68%)

Conclusions: The proposed method allows identifying adverse drug reactions from user reviews and social media posts in English and Russian. The obtained results showed the necessity to develop the linguistic resources to improve the quality of classification.

Acknowledgment: Supported by Russian Government Program of Competitive Growth of KFU, funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7).

P129-F | Assessing the quality of newly formed bone tissue using scanning electron microscopy

A. Khairutdinova; I. Khafizov; Y. Osin; A. Rizvanov; I. Sharafutdinov; R. Khafizov
Kazan Federal University, Kazan, Russian Federation

Background: Current approaches only partially resolve the bone augmentation tasks. Thus, they increase the bone volume without any account of its quality. This study is focused on bone development in ridge-deficient areas using stem cells isolated from the stromal vascular fraction of the dog fat tissues in combination with porous titanium-nickelide granules and a porous membrane.

Material and methods: Samples of formed bone blocks were obtained after 1, 3 and 6 months. The experiments were performed in biological triplicates with 3 repeats in each run. Samples were fixed and placed in the chamber of Quorum Q 150T ES vacuum coater. The conductive layer was applied by cathode sputtering using Au/Pd conductor paste with ratio of 80/20. All measurements were performed with a Merlin high-resolution field emission scanning electron microscope (Carl Zeiss) equipped with AZtec X-Max energy-dispersion spectrometer at resolution 127 eV. The probing depth was of 1 μm, 20 measurement per sample were performed.

Results: Calcium and phosphorus ratio was registered in the bone samples. Calcium distribution was unequal at different time points. Normal Ca/P ratio in bone tissue is 2:1. We observed the ratio close to 1:1 after 1–3 months. Although 6 months-old samples demonstrated an uneven distribution of chemical elements, the balance between Ca and P was maintained in most cases (2:1), suggesting high quality of the newly formed bone in the submembrane space and around titanium nickelide granules.

Conclusions: The use of stromal vascular fraction from fat tissues in combination with porous titanium nickelide granules favors and promotes the formation of fully developed bone tissue which is important for clinical implantology.

This work is supported by Russian Government Program of Competitive Development of Kazan Federal University.

P130-F | Different performance of mating behavior in white mutant of Drosophila during the aging

V. Kostenko; I. Sharafutdinov; A.G. Porfiriev
Kazan Federal University, Kazan, Russian Federation

Background: Aging is an irreversible process accompanying by a decrease in reproductive function and fertility. The age-related changes involve serious alterations in reproductive machinery of the organism causing significant impact on the offspring. This research was aimed to investigate the impact of early neurodegenerative processes on mating behavior on the Drosophila model system.

Material and methods: Wild-type Canton-S flies and mutant line white1 (pigment-free eyes) with and decreased level of serotonin and dopamine were used. Mating activity (MA) of males and female sexual receptivity (FR) were estimated by the number of males and females involved in mating within one hour in five independent experiments. The females and males ($n = 250 + 250$ per experiment) were kept separately before the behavioral test. The dynamics of age-related changes were observed within 21 days of Drosophila adult life. The significance of differences between experimental groups was assessed by using Factorial ANOVA test and Student's *t*-test.

Results: In 3 days-old wt flies, the percentage of MA and FR were 64 ± 4.57 and 77 ± 5.82 respectively. In mutant w1 flies these parameter were significantly lower (25 ± 3.7 of MA and 43 ± 3.1 of FR). While no significant differences on both mating traits on 3nd and 18th days was observed for wt flies, MA and FR of w1 flies decreased twice from 9th day (13 ± 2.16 and 18 ± 2.91, respectively). Since the changes of behavioral are associated with suppression of the nervous system, a histological analysis of imago brain was performed to evaluate the degenerative changes during aging. Our data show that the pronounced degeneration occured mainly in mushroom bodies and lobula responsible for behavior of flies in the w1 line.

Conclusion: The behavioral differences during aging between white mutant and wild-type flies are caused by degenerative changes of neurons in mushroom body and lobula of the brain of white mutants occurring in earlier age. The physiological background of these changes apparently is linked with decreased level of serotonin and dopamine in the brain during aging.

This work is supported by Russian Government Program of Competitive Development of Kazan Federal University.

P131-F | Acrolein detection by in vivo synthetic chemistry: unexplored reactivity of acrolein with azide

A. Pradipta*; E. Saigitbatalova†; A. Kurbangalieva†; K. Tanaka*,†

Biofunctional Synthetic Chemistry Laboratory, RIKEN, Wako-shi, Saitama, Japan; †Biofunctional Chemistry Laboratory, A. Butlerov Institute of Chemistry, Kazan Federal University, Kazan, Russian Federation

Background: Acrolein, a highly toxic α,β-unsaturated aldehyde, has been a longstanding key biomarker associated with a range of disorders related to oxidative stresses. Currently available analytical methods rely on the indirect protocols, e.g., derivatization/HPLC or mAb detection of the lysine adducts. Developing new analytical tools for acrolein detection that are straightforward, cost-effective, selective, and preferably feasible using live cells remains a highly essential pursuit in the diagnosis and therapeutic treatment of oxidative stress-related diseases.

Material and methods: Three systems were investigated using acrolein labeling approach. Human umbilical vein endothelial cells were treated with solution of TAMRA-labeled azides at room temperature for 30 minutes, accompanied by [1] pretreatment with excess acrolein; [2] exposure to tobacco smoke; and [3] the presence of hydrogen peroxide, which induced cellular oxidative stress. The cells were fixed by paraformaldehyde and analyzed by laser scanning confocal microscopy.

Results: We demonstrated that for the first time aryl azides can rapidly and selectively react with acrolein in a "click" manner to provide 4-formyl-1,2,3-triazolines and 4-formyl-1,2,3-triazoles. The azide-acrolein conjugates were found not to be cytotoxic under the experimental conditions, based on the cellular morphologies. When treating a fluorescently labeled phenyl azide with oxidatively stressed or smoking-associated cell models, these heterocyclic compounds could be selectively taken up by the cells and preferably localized at the endoplasmic reticulum and lysosome, leading to a new convenient tool for both effectively detecting acrolein level and directly imaging live cells that are under stress.

Conclusions: We developed a new method for detecting and imaging acrolein extracellularly released by cells in the context of oxidative stress processes or introduced via environmental exposure.

Acknowledgments: This work was supported by the Russian Government Program of Competitive Growth of Kazan Federal University, JST PREST, JSPS KAKENHI Grant Numbers JP16H03287, JP16K13104, and JP15H05843 in Middle Molecular Strategy.

P132-F | Therapeutic in vivo synthetic chemistry: exploring on opportunity to activate drugs at specific sites in the body

K. Tsubokura*; K. Vong*; R. Sibgatullina†; A. Kurbangalieva†; K. Tanaka*,†

Biofunctional Synthetic Chemistry Laboratory, RIKEN, Wako-shi, Saitama, Japan; †Biofunctional Chemistry Laboratory, A. Butlerov Institute of Chemistry, Kazan Federal University, Kazan, Russian Federation

Background: The use of metal complex catalysts within biological systems is mostly limited to cell and bacterial systems. Nowadays, metal-catalyzed chemical reaction within mature mammals captures the significant attention. Moreover, the site-specific reaction is more attractive in terms of developing drug delivery systems.

Material and methods: The gold-ion catalyst was linked via an intermediate locking system to an albumin, which was tagged with dozen glycan molecules. It was introduced into 8 to 10-week-old BALB/cAJcl-nu/nu mice via the tail vein ($N = 6$). After 30 minutes, fluorescently labeled propargyl ester probe was injected, abdominal side and dorsal images were taken at 30-minute intervals.

Results: The presence of glycan markers on albumin surface have led to the Au(III) complexes accumulation at targeted organs (liver or intestine) without leaching or deactivation of the metal catalysts. Subsequent

fluorescently labeled propargyl ester probe introduction resulted in the target-selective gold-catalyzed amide bond formation between propargyl ester probes and amines on surface proteins. It was proved by the fluorescence ratios of the targeted organs based on the region of interest within a whole body 2 hours after fluorescent probe administration. As a control, mice were also treated with the gold-deficient glycoalbumin complex and propargyl ester, where the probe was immediately distributed over the whole body.

Conclusions: The first example of transition-metal-catalyzed bond formation selectively at targeted organs within a live animal is reported. It was shown that gold complexes can be delivered to target organs in living mice, where they can speed chemical reactions for diagnostic or therapeutic purposes. This method enables various therapeutic molecules to be synthesized directly at the target organs in living organisms.

Acknowledgments: This work was supported by the Russian Government Program of Competitive Growth of Kazan Federal University, JST PREST, JSPS KAKENHI Grant Numbers JP16H03287, JP16K13104, and JP15H05843 in Middle Molecular Strategy.

P133-F | Development of a panel of pancreatic cancer cell lines expressing doxycycline inducible spCas9

V. Skripova*; A. Nurgalieva*; I. Astsaturov*,†; R. Kiyamova*

Kazan Federal University, Kazan, Russian Federation; †Fox Chase Cancer Center, Philadelphia, USA

Background: Pancreatic cancer (PC) is one of the most aggressive types of cancer with high lethality rate due to multiple chemoresistance that has been developing in most cases. Understanding of chemoresistance mechanisms is critical to develop new effective treatment strategies for PC patients. Previously we applied primary drop-out genetic CRISPR/Cas9 screening of pancreatic cancer AsPC-1 cells expressing Cas9 and sgRNA libraries targeting whole-genome and cell-cycle genes to identify genes regulating platinum resistance. We identified 130 genes knock-out of which significantly changed platinum sensitivity (Skripova et al., 2016). In this work we created the panel of PC cell lines expressing doxycycline inducible spCas9 in order to validate 130 nominated genes using newly synthesized focused sgRNA library.

Material and methods: PC cell lines AsPC-1, Panc-1, CFPAC-1, HPAF-II, MIA PaCa-2, BxPC-3 and Capan-2 were transduced by lentivirus containing Lenti-iCas9-neo plasmid encoding a doxycycline inducible of FLAG-tagged spCas9. 0.5 mg/mL of G418 was used to select transduced cells. Western blot analysis with anti-FLAG-epitope primary antibody was used to detect Cas9 expression.

Results: Transduced cells were selected with G418 and single cell clones of each line were obtained. 7–12 clones per each cell line were checked for Cas9 expression after 6 days incubation with 1 μg/mL of doxycycline. 30–95% of clones showed Cas9 expression. Clones with the highest Cas9 expression level were chosen for further work.

Conclusion: Panel of PC cell lines expressing spCas9 including AsPC-1, Panc-1, CFPAC-1, HPAF-II, MIA PaCa-2, BxPC-3 and Capan-2 was created. Created panel is useful for further CRISPR/Cas9 based researches. The panel will be used for validation of 130 genes identified in our previous work as well as for deeper investigation of contribution of individual validated genes in platinum resistance mechanisms.

Acknowledgments: Work was supported by Russian Science Foundation (project no. 15-15-20032) and Program of Competitive Growth of KFU.

P134-F | The effect of small molecule compounds Physcion and PFI-3 on the sensitivity of the tumor cell lines SCC61 and AcPC-1 to cisplatin

D. Savenkova; L. Minigulova*; K. Havrysh*; V. Skripova*; I. Serebriiskii*,†; R. Kiyamova*

Kazan Federal University, Kazan, Russian Federation; †Fox Chase Cancer Center, Philadelphia, USA

Background: One of the main problems of modern oncology is the resistance of tumors to chemotherapeutic drugs, including cisplatin. One of the most promising approaches to overcome drug resistance is combination therapy. This study was designed to investigate the sensitivity of pancreatic AsPC-1 and head and neck SCC61 cancer cell lines to cisplatin in combination with small-molecule compounds named Physcion [R. Lin, 2015] and PFI-3 [B. Vangamudi, 2015] which are inhibitors of the 6-phosphogluconolactonase (PGD) and bromodomains of SMARCA2/SMARCA4, respectively.

Methods: SCC61 and AsPC-1 cancer cell lines were cultured in 96-well plates (4 000 cells per well) and treated with cisplatin (1–128 μM) in combination with Physcion (10 and 25 μM) and PFI-3 (25 and 50 μM). The experiment was repeated in 3 technical and 3 biological replicates. The viability curves were constructed and IC50 values were determined. The level of statistical significance was determined using the Fisher criterion.

Results: It was shown that both 10 and 25 μM of Physcion significantly ($P < 0.05$) decreased cisplatin IC50 for SCC61

cells by 18% and 13%, respectively. Cisplatin IC50 of AsPC-1 cells was significantly ($P < 0.05$) decreased by 30 % in combination with 10 μM of Physcion. Both 25 and 50 μM of PFI-3 significantly ($P < 0.05$) increased cisplatin IC50 of both cell lines by 28% and 65% respectively.

Conclusion: Inhibition of PGD and SMARCA2/SMARCA4 bromodomains led to an increase and decrease of cisplatin sensitivity of both SCC61 and AsPC-1 cell lines, respectively. These observations could be an evidence of PGD and SMARCA2/SMARCA4 role in cisplatin sensitivity regulation in cancer cells.The obtained data could be used for further investigation of molecular mechanisms of the cancer cell chemotherapy sensitivity/resistance which would have a practical importance in future.

Acknowledgments: Work was supported by Russian Science Foundation (project no. 15-15-20032) and Program of Competitive Growth of KFU.

P135-F | Adaptation of *Acholeplasma laidlawii* to adverse environments as well as antibiotics is accompanied by multiple genome mutations, transfer of mutant genes through extracellular vesicles and mutagenicity to human cells

E. Medvedeva*,†; D. Khusnutdinova†; N. Baranova*,†; T. Malygina*; M. Dramchini†; A. Mouzykantov*,†; M. Davydova*; O. Chernova*,†; V. Chernov*,†

**KIBB – Subdivision of FIC KazanSC of RAS, Kazan, Russian Federation; †Kazan (Volga Region) Federal University, Russian Federation*

Background: *Acholeplasma laidlawii* (class Mollicutes) is the main contaminant of cell cultures and vaccine preparations, being a danger to human health. The solution to the problem of eradicating the mollicute is associated with the elucidating molecular mechanisms of adapting the microorganism to stressors, including antimicrobials. The comparative analysis of the complete genomes and virulence of the *A. laidlawii* PG8B strains adapted to adverse environments and antibiotics was the objective of the study.

Material and methods: We selected *A. laidlawii* strains with differential sensitivity to long-term exposure to low temperature, substrate limitation, ciprofloxacin and tetracycline. Genomes of all strains were sequenced by different methods (454 Roche JS Junior, MiSeq, NextSeq). The evaluation of mutagenicity was performed on human peripheral blood lymphocytes. Three replicates of the experiments were conducted. Statistical analysis was performed using chi-squared test.

Results: As a result of genomic analysis of *A. laidlawii* strains we have found that adaptation of the mollicute to the stressors is accompanied by multiple genome mutations associated with genes coding proteins involved in the fundamental cellular processes and targets for antimicrobials of different classes, including those indifferent for these bacteria. The mutant genes were detected in the *A. laidlawii* extracellular vesicles, which could provide gene distribution in bacterial populations via horizontal transfer. A considerable part of the mutations occurred in virulence genes. It was found that the strains adapted to the stressors induced total premature centromere separation in human lymphocytes in vitro.

Conclusions: Adaptation of *A. laidlawii* to adverse environments and antibiotics is accompanied by multiple genome mutations, transfer of mutant genes through extracellular vesicles and mutagenicity to human cells.

The work is supported by the Grant of President of the Russian Federation MK-1099.2017.4, grant RFBR 18-04-00660, the Russian Government Program of Competitive Growth of KFU.

P136-F | Influence of ultraviolet irradiation and ADSCs on the regenerative properties of the skin

G. Masgutova; V. Syromiatnikova; M. Gomzikova; A. Mullakhmetova; R. Masgutov; A. Rizvanov

Kazan (Volga Region) Federal University, Kazan, Russian Federation

Background: Adipose-derived stem cells (ADSCs) represent a promising perspective for regenerative medicine. Morphometric parameters of the skin analysis after UV-irradiation exposure followed by ADSCs therapy were conducted.

Materials and methods: Adult white mice with body mass 20–30 g, ($n = 28$) were divided into 3 control and 1 experimental groups: intact ($n = 7$), depilation ($n = 7$), UV-group ($n = 7$) and UV + ADSCs group ($n = 7$). Animals were daily depilated and subjected to UV irradiation (20–40 minutes) for 6 weeks. After 6 weeks in experimental group ADSCs were intracutaneously injected (1 million cells in 500 μL PBS) into the irradiated skin. Four weeks later the dorsal skin of the mice were fixed in 10% formaldehyde and embedded into the paraffin. The transverse sections were stained with hematoxylin and eosin.

Results: Histological analysis showed a decrease of the thickness of epidermal layer of skin in a group of mice that underwent regular depilation compared to the skin of intact animals (24.77 ± 13.52 μm vs 17.16 ± 7.3 μm recpectively). In group UV + ADSCs, the thickness of epidermal layer of skin was reduced compared to the UV-group (25.24 ± 7.66 μm vs 18.38 ± 7.68 μm respectively). The thickness of the dermis under UV influence increased in

comparison with intact group (329.85 ± 48.68 μm), but in the group with ADSCs therapy the thickness of the dermis was reduced in comparison with UV-group (242.73 ± 41.45 vs 301.31 ± 90.38 μm respectively).

Conclusion: UV rays are responsible for skin changes, such as epidermal and dermal thickening. We can suggest that adipose derivate stem cells therapy is promising for the treatment of injured skin. Further studies are required.

The Russian Government Program of Competitive Growth of Kazan Federal University supported this study. State assignment 20.5175.2017/6.7 of the ministry of Education and Science of the Russian Federation supported Albert Rizvanov.

P137-F | Specificity of microvesicles interactions with target cells

M. Gomzikova; S. Kletukhina; S. Kurbangaleeva; O. Neustroeva; A. Rizvanov

Kazan (Volga Region) Federal University, Kazan, Russian Federation

Background: Microvesicles (MVs) are membrane spherical structures which are capable of carrying biologically active molecules. Extracellular vesicles' membrane receptors are expected to participate in recognition and specific binding with the surface proteins of target cells. Thereby, MVs can be used as vector system for targeted delivery of drugs. Cytochalasin B induced membrane vesicles (CIMVs) were used as a vector for nanoparticles, dye and chemotherapeutic drugs delivery. However, the specificity of CIMVs interaction with target cells has not been studied.

Materials and methods: CIMVs were obtained from PC3 cells. Four different cancer cell lines were taken as recipients: PC3, SH-SY5Y, HCT116, HeLa. The size of obtained CIMVs and specificity of fusion with recipient cells were evaluated by flow cytometry (BDFACS Aria III) and laser confocal microscopy (Carl Zeiss LSM 780). Results: We found that the majority of CIMVs obtained from PC3 cells has size less than 220 nm and up to 1340 nm (95% of CIMVs). We found that fusion efficiency of PC3 CIMVs with SH-SY5Y, PC3, and HCT116 cell lines wasn't significantly different (% of cells containing CIMVs membrane component was 59.46 ± 3.8%, 56.81 ± 0.41%, 58.95 ± 3.9% respectively). Proteinase K treatment decreased CIMVs integration in PC3 cells by 33.8 ± 6.3 %, SH-SY5Y cells by 54.8 ± 4.97%, HCT116 cells by 51.4 ± 1.76%, HeLa cells by 85.6 ± 4.2% compared to positive control (cells incubated with CIMVs at 37°C in full medium).

Conclusions: We found that there is no significant preferences in CIMVs fusion with target cells of the same type (homophilic membrane proteins interaction). Disruption of surface receptors had greatest impact on PC3 CIMVs penetration into target cells, implying that heterophilic interaction is more significant in the process of recognition and fusion of extracellular vesicles with target cells. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

P138-F | Biodistribution of membrane vesicles in vivo

M. Gomzikova; O. Neustroeva; S. Kletukhina; S. Kurbangaleeva; M. Mavlikeev; A. Rizvanov

Kazan (Volga Region) Federal University, Kazan, Russian Federation

Background: Extracellular vesicles (EVs) are important vehicles carrying growth factors, cytokines, chemokines, mRNA, miRNAs and siRNA which mediate intercellular communication. EVs contain the same bioactive molecules and surface receptors similar to donor cells. These properties suggest that membrane vesicles might be a perspective therapeutic instrument instead of mesenchymal stem cells (MSC), which have risk of tumor growth. Therefore, we investigated the biodistribution of allogeneic membrane vesicles in vivo after subcutaneous and intramuscular injection in mice.

Materials and methods: We obtained cytochalasin B-induced membrane vesicles (CIMVs) from adipose tissue-derived mouse stem cells (ADSCs) and stained with fluorescent membrane dye DiD (ThermoFisherScientific, USA). Allogeneic CIMVs were injected subcutaneously and intramuscularly in three mice at two different concentrations 1 mg/mL and 0.5 mg/mL. Fluorescence signal was detected in vivo using IVIS Spectrum (PerkinElmer, USA) (3 measurements per mouse).

Results: After subcutaneous administration the fluorescence intensity of 0.5 mg/mL CIMVs was 2.705 relative fluorescence units, fluorescence intensity of 1 mg/mL CIMVs – 5.534 relative fluorescence units (through 1 hour). We were able to detect CIMVs injected subcutaneously and intramuscularly after 1 hour, 48 hours and even 14 days. According to the 3D modeling, subcutaneous injection was localized under the skin surface, and intramuscular injection led to the CIMVs spreading and fluorescence signal was located at different focal lengths.

Conclusions: The fluorescence intensity of the 1 mg/mL CIMVs was twice greater than the 0.5 mg/ml CIMVs that confirms the specificity of the fluorescent signal. Subcutaneous and intramuscular administration of membrane vesicles derived from MSC may be useful for the therapy of diseases, such as skin damage, lower limb ischemia and

others. CIMVs may be used not only for the transfer of bioactive molecules but also for drug delivery to different tissue. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

P139-F | Modelling reflex arc for a memristive implementation

M. Talanov*; I. Lavrov*,†; V. Erokhin*,‡

*Kazan Federal University, Kazan, Russian Federation; †Mayo Clinic, Rochester, USA; ‡Institute of Materials foe Electronics and Magnetism, Italian National Council of Research, Parma, Italy

Background: We introduce principal schematic for a reflex arc to be implemented via electronic memristive device. The reflex arc is well understood, researched part of a nervous system and already was implemented as several electronic devices. The main advantage of the proposed approach is adaptability and self-learning options that are achieved using novel electronic elements memristors or memristive devices. Previously it was demonstrated by one of our co-authors that polyaniline memristive devices are capable of biological like STDP learning in the system of two biological neurons connected only via a memristive device. Using this effect we hope to recreate the dynamics of adaptivity of a reflex arc as an electronic device that could be used as neuromorphic prosthesis.

Model: We present the computational model of a reflex arc implemented based on works of Igor Lavrov and Marco Capogrosso. The model is dedicated to demonstration of basic processes of neuronal activity of a reflex arc. The modelling is done for the purposes of validation and further development of electronic schematic that could be used as a neuromorphic prosthesis device. We have used two modelling engines to indicate the plausibility of the model: Neuron and NEST and in both cases we have implemented same schematic with different level of details: neuronal and synaptic in NEST and multi-compartmental taking in account dendrite tree topology in Neuron.

Results: We present the simulation results via two neurosimulators NEST and NEURON that could be used for validation of the computational model in comparison with biological experimental results. We run series of computational experiments 30 on KFU university cluster. We indicate the evolution of bio-plausibility gradually optimising the topology of proposed neuronal circuitry of the proposed model. To indicate the bio-plausibility during the validation we have started with most primitive circuitry evolving to bio-plousible model that indicate the neuronal activity and dynamics matching biological models and experimental results. Later we experimented adding noise to match the bio-plausible channel activity dynamics.

Conclusion: We present the computational model and the validation via simulation of a reflex arc and the comparison with biological experimental results. The model and simulation is developed for further development of a reflex arc neuromorphic prosthesis.

Work supported by Program of Competitive Growth of KFU and was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities number 2.8303.2017/8.9.

P140-F | Bio-plausible model of electronic memristive neuron

M. Talanov*; E. Zykov*; I. Lavrov*,†; V. Erokhin*,‡

*Kazan Federal University, Kazan, Russian Federation; †Mayo Clinic, Rochester, USA; ‡Institute of Materials foe Electronics and Magnetism, Italian National Council of Research, Parma, Italy

Background: There were several models of biological neuron developed so far for different purposes, starting from famous McCulloch and Pitts that is currently widely used in artificial intelligence applications, Izhikevich and Hodgkin–Huxley models. The interest to new models of biological neuron is increased by huge projects: the Human Brain Project, Blue Brain Project and the BRAIN initiative as well as the exponential interest to almost all spheres of the artificial intelligence.

Model: In this study we introduce high-level design schematic and block diagram of a memristive electronic neuron that is capable of three types of STDP learning: excitatory: Hebbian or 1 x, inhibitory: "sombrero" and sinusoidal like function. The proposed schematic is also capable of neuromodulation via dopamine D1 receptor that modulates the amplitude of learning functions. The proposed high-level black diagram of a memristive neuron device is designed to be used in neuromorphic prosthesis devices in invasive and non-invasive manner, as well as exoskeletons and robotic devices.

Results: The modelling results are presented in form of simulation of electronic schematic via LTSpice tool as well as physical prototyping of parts of a schematic. We demonstrate the results of modelling and validation that plays important role to indicate an option of a memristive device to be integrated with biological nervous system. For validation purposes we run the series of experiments 30 to test the functionality and demonstrate the bio-signals compatibility taking in account the timing parameters and dynamics of spikes.

Conclusions: In this study we propose novel architecture of bio-plausible memristive neuron that is capable of inhibition and neuromodulation via D1 dopamine receptor. We demonstrate results of validation of the model via simulation with the comparison with biological neurons spiking activity. We plan to use the results of the current project results during the electronic implementation of a memristive reflex arc that could possibly be used as neuromophic prosthesis for artificial or natural limbs.

Work supported by Program of Competitive Growth of KFU and was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities number 2.8303.2017/8.9.

P141-F | Computational modeling of spinal locomotor circuitry

M. Talanov*; I. Lavrov*,†; C. Menshenin*

Kazan Federal University, Kazan, Russian Federation; †Mayo Clinic, Rochester, USA

Background: Lumbosacral segments of the spinal cord are isolated from a mammalian brain, they can produce locomotor pattern in the hindlimbs via pharmacological and/or electrical spinal cord stimulation. Although the mechanisms for this phenomenon have been extensively investigated, there is still no explanation for the complexity of the motor programs generated by the spinal cord networks.

Materials and methods: Recently we have developed a novel electrophysiological assessment for evaluation of selective spinal cord evoked responses (earlier, middle and late responses). Modulation of these responses during different tasks can indicate on mechanisms of spinal cord neuromodulation and organization of spinal network. Using this approach, we designed a model of spinal circuitry responsible for generating of the stepping pattern and implemented is using neurosimulator NEST. With the simulation time 20 seconds, each computation took approximately 11 minutes using 8 cores of CPU.

Results: We propose a novel multilevel model of spinal circuitry based on our experimental results. We have found that computer model produces similar to biological results outcome with some variability, which can be explained by variation in proposed topology and in sensory input. During simulation in silica we observed systematic modulation of monosynaptic and polysynaptic responses similar to in vivo results, which represent different components of spinal circuitry. For the validation purposes we have run series of experiments 10 000 to indicate overall plausibility of the proposed circuitry as the locomotor pattern generator and we could indicate overall dynamic characteristics matching with research initially biological model.

Conclusions: Observed similarity suggests that proposed network organization has relevance to biological network responsible for complex patterns like stepping, and that proposed model could provide a new insight into dependent modulation of different levels of spinal circuitry responsible for locomotion. As a next step, we plan to further validate computer model by comparison with in vivo experimental data and also implement proposed topology to recreate electric circuitry for partial spinal cord segment prosthesis.

Work supported by Program of Competitive Growth of KFU and was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities number 2.8303.2017/8.9.

P142-F | Virtual Reality-Based Immersive Simulation for Invasive Surgery Training

V. Kugurakova*; V. Abramov*; R. Sultanova*; I. Tsivilskiy†; M. Talanov*

Kazan Federal University, Kazan, Russia; †Kazan National Research Technical University named after A. N. Tupolev – KAI, Kazan, Russia

Background: Currently, the curriculum of higher medical education in the study of medical students in various sections of surgery, primarily classical basic approaches to invasive surgical intervention, includes not only theoretical studies in classes, followed immediately by practical lessons in morgues and hospitals. To support these educational processes in medical surgery, we are developing a realistic and a gaming approach using virtual reality.

Materials and methods: Hand Tracking technology allows you to track not only your palms, but your fingers, and even their phalanges, up to 22 points on each hand. The Full Hand mode also returns all the information about the joints, their position, and even rotation, and also recognize the gestures. Thus, this technology will allow you to sharpen the accuracy of the movement of your fingers during operations, if you add the control to the correctness of the actions, including the depth of the cut. Recreating the accuracy of anatomical structures and the mechanics of interaction is one of the most important tasks.

Results: Our solution allows us to simulate the realistic behavior of body tissues, so pressing, stretching, cutting, sewing and welding processes of human tissues become realistic. A special realism to the educational process is the increase in immersion through the realization of a realistic environment of the surgical operating room.

Increasing of immersivity will lead to developing the pre-surgery experience that will help a student in real surgery practice.

The developing system can make all simulation unique by randomization patient parameters such as sugar level or blood coagulability and etc. but also teacher have the ability to customize these parameters in reasonable variety.

Conclusions: We present a new immersive approach to teach surgery students with virtual reality technology and hand tracking with force feedback.

Work supported by Program of Competitive Growth of KFU and was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities number 2.8303.2017/8.9.