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ABSTRACT

SYMPOSIUM 1 – MITOCHONDRIA

57ASM – 007 | Dysfunction of endoplasmic reticulum-mitochondria contacts in bipolar disorder

A.C. Pereira[1]; A. P. Marques[1]; R. Resende[1]; L. Serrano-Cuñarro[1]; M. Batista[2]; A. Macedo[3]; Cl. Pais[4]; J. B. Melo[4]; N. Madeira[3]; M. T. Cruz[1]; C. Cavadas[1]; C. Pereira[1]

[1]CNC-Center for Neuroscience and Cell Biology, Coimbra, Portugal; [2]CHUC-Centro Hospitalar e Universitário de Coimbra, Department of Dermatology, Coimbra, Portugal; [3]CHUC-Centro Hospitalar e Universitário de Coimbra, Department of Psychiatry Coimbra, Coimbra, Portugal; [4]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Introduction: Mitochondria-Associated Membranes (MAMs), which are the contact sites between the endoplasmic reticulum (ER) and mitochondria, are dynamic platforms that regulate several key processes, particularly mitochondrial bioenergetics and dynamics, as well as stress responses, due to the transfer of Ca2+ and lipids between both organelles. MAMs are modulated according to the cellular needs, and the impairment of these stress response structures has been linked to pathological conditions closely associated with mitochondrial dysfunction and ER stress, among other deleterious events. Since these molecular alterations have been closely associated with the structural brain changes and cognitive deficits reported in Bipolar disorder (BD), this study aimed to investigate the role of MAMs in BD physiopathology.

Materials and Methods: Dermal fibroblasts from BD patients versus healthy matched controls were used as an in vitro model of BD. The ER-mitochondria coupling was assessed by confocal microscopy and transmission electron microscopy. Concomitantly, MAMs functional parameters such as Ca2+ fluxes, reactive oxygen species (ROS) production, and lipid droplets formation, were determined using the Fluo-4/Rhod-2, Mitopy/CellROX and LipidTOX fluorescent probes, respectively. Mitochondrial bioenergetics were evaluated by the Seahorse assay. Protein levels of ER stress-induced Unfolded Protein Response (UPR) markers were assessed by Western Blot, and autophagy was investigated by measuring the immunoreactivity of the autophagy-associated protein LC3B.

Results: In BD patients-derived fibroblasts, it was observed an increase in the number of close ER-mitochondria contacts sites, or MAMs, which was shown to be correlated with functional alterations, such as deregulated ER-to-mitochondria Ca2+ transfer, enhanced ROS production, ER-to-mitochondria lipids transfer and formation of Lipid droplets, ER stress induction, as well as perturbation of mitochondrial metabolism and autophagy.

Conclusion: This study shows that the impaired ER-mitochondria communication at MAMs is implicated in BD physiopathology and might impact several crucial cellular and molecular events leading to the clinical manifestations of the pathology.

57ASM – 013 | Mitochondria in intercellular communication between stem and immune cells

V. Somova; B. Porubská; M. Krulová

Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

Introduction: Stem cells have considerable potential to modulate the immune system. Besides paracrine functions, mitochondrial transfer is an important mechanism through which stem cells reduce inflammation by balancing cellular stress. Furthermore, by supporting mitochondrial maintenance and biogenesis, stem cells could induce metabolic switch and regulate the phenotype of immune cells. We previously confirmed the ability of different types of stem cells to transfer mitochondria to immune cells [1]. However, the precise mechanism underlying mitochondrial transfer is yet to be discovered. By describing factors responsible for nanotube formation or mitochondrial morphology suitable for the transfer, we aim to explain the regulatory mechanism behind intercellular mitochondrial dynamics.

Methods: In this study, a mitochondrial transfer between stem and spleen cells isolated from BALB/c mice was analysed in vitro. Samples from >3 independent experiments were measured using flow cytometry, and RT-qPCR and compared using one-way ANOVA. Bulk RNAseq was performed to evaluate the differences in the transcriptional profile of acceptors and non-acceptors of mitochondria.

The mitochondrial morphology and dynamics between stem and immune cells were also visualized by fluorescence microscopy.

Results: Our study confirmed that stem cells transferred mitochondria to various leukocyte populations with different efficiency and mitochondria acceptors showed prolonged survival. Various mechanisms, including mitophagy, cell stimulation, and ROS production, played an essential role in this process.

Conclusions: Stem cells transfer mitochondria to different immune cell populations with different potency, least frequently to B cells. Interestingly, processes associated with immune cell activation promoted mitochondrial transfer. Thus, we hypothesize that mitophagy and ROS production associated with immune cell priming are important mechanisms underlying mitochondrial transfer efficacy.

References

1. Porubska B, Vasek D, Somova V, et al. Sertoli Cells Possess Immunomodulatory Properties and the Ability of Mitochondrial Transfer Similar to Mesenchymal Stromal Cells. Stem Cell Rev Rep. 2021;17(5):1905–1916. doi:10.1007/s12015-021-10197-9.

57ASM – 032 | High-resolution respirometry is comparable in 0.5 mL and 2.0 mL chamber volumes: Studies with platelets, permeabilized fibroblasts, and isolated mitochondria

E. Leo[1]; L. Zdrazilova[1,2]; L. F. Garcia-Souza[1]; E. Åsander-Frostner[3]; E. Elmér[3]; E. Gnaiger[1]
[1]Oroboros Instruments, Innsbruck, Austria; [2]Department of Paediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General Hospital, Prague, Czechia; [3]Mitochondrial Medicine, Lund University; Abliva AB, Lund, Sweden

Introduction: The volume of the experimental chamber is a fundamental feature in the design of respirometric instruments applied in mitochondrial and cell physiology. Small volumes are considered to minimize the amount of tissue or cell count required, but increasing problems are encountered of O_2 back diffusion, homogenous stirring, and scatter in small-volume wells [1].

Material and Methods: We evaluated instrumental performance and respirometric results obtained in parallel experiments with high-resolution respirometry using experimental chamber volumes of 2.0 mL and 0.5 mL. Instrumental background tests performed revealed the expected volume-dependent differences. Human platelets, human permeabilized fibroblasts, and mouse cardiac isolated mitochondria were studied by substrate-uncoupler-inhibitor titration (SUIT) protocols and the correlation between the two chambers was calculated by inverted regression analysis [1].

Results: Close to 4-fold lower amounts of sample were required in 0.5 mL compared to 2.0 mL chambers. In all biological models, oxygen fluxes in the two chambers were highly correlated, with $r^2 = 0.99$ in platelets; 0.96 and 0.95 in fibroblasts applying two SUIT protocols; and 0.99 in cardiac mitochondria. The general offset from identity was less than 10% at high fluxes and negligible at very low ones.

Conclusions: In studies with cells and isolated mitochondria, application of the 0.5 mL module is advantageous for reducing the amount of sample, with identical results obtained in the 2 mL chamber. A wet mass <0.5 mg of permeabilized fibres suitable for the small-volume chamber, however, is difficult to be determined accurately. Application of the 0.5 mL chamber under hyperoxia or deep hypoxia remains to be evaluated.

References

1. Zdrazilova L, Hansikova H, Gnaiger E (2022) Comparable respiratory activity in attached and suspended human fibroblasts. PLoS ONE 17:e0264496. https://doi.org/10.1371/journal.pone.0264496

57ASM – 034 | The cytochrome c test for respirometric quality control of the integrity of the mitochondrial outer membrane

A. Timón-Gómez; E. Baglivo; E. Gnaiger
Oroboros Instruments GmbH, Innsbruck, Austria

Introduction: Cytochrome c is the redox link between Complexes CIII and CIV of the mitochondrial electron transfer system. Cytochrome c release across the mitochondrial outer membrane (mtOM) is associated with apoptosis. Stimulation of respiration by added cytochrome c indicates damage of the mtOM. This respirometric test for mtOM integrity provides a quality control to exclude potential injuries caused experimentally during mitochondrial preparation by tissue homogenization or plasma membrane permeabilization.

Aim: Selecting the step of cytochrome c addition in substrate-uncoupler-inhibitor-titration (SUIT) protocols is of primary importance for diagnostic analysis. This study presents a quality control of SUIT protocols using the cytochrome c test.

Methods: We applied SUIT protocols RP1 and RP2 to address the cytochrome c effect in different respiratory states. The first steps in RP1 use pyruvate & malate to analyse

coupling control in the NADH-pathway. RP2 focuses on fatty acid oxidation (FAO), considering malate-linked anaplerosis. We tested these protocols in HEK 293 T permeabilized cells and beef liver and heart homogenate.

Results: Cytochrome c control efficiency [1] was correlated when cytochrome c was added in the ADP-activated OXPHOS state of the NADH-pathway and in FAO. Following 0.1 mM malate in RP2, addition of cytochrome c before or after titration of fatty acid did not affect the O2 flux in FAO. Optimization of digitonin concentrations for plasma membrane permeabilization of cultured cells was essential to avoid damage of the mtOM.

Conclusions: Underestimating respiratory capacities in mitochondrial preparations is avoided by adding cytochrome c as an early step of SUIT protocols. However, cytochrome c added in the LEAK state causes an unexplained activation of respiration.

[1] Gnaiger E (2020) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. https://doi.org/10.26124/bec:2020-0002

57ASM – 051 | Stress-dependent macromolecular crowding in the mitochondrial matrix

W. J. H. Koopman

Department of Paediatrics, Radboudumc, Nijmegen, The Netherlands

Macromolecules of various size induce crowding of the cellular environment. This crowding impacts on biochemical reactions by increasing solvent viscosity, decreasing the water-accessible volume and altering protein shape, function and interactions. Although mitochondria represent highly protein-rich organelles, most of these proteins are somehow immobilized. Therefore, it is still unclear whether the mitochondrial matrix solvent exhibits macromolecular crowding. Here, we demonstrate that fluorescent protein fusion peptides (AcGFP1 concatemers) in the mitochondrial matrix of HeLa cells display an elongated molecular structure and that their diffusion constant decreases with increasing molecular weight in a manner typical of macromolecular crowding. Chloramphenicol treatment impaired mitochondrial function and reduced the number of cristae without triggering mitochondrial orthodox-to-condensed transition or a mitochondrial unfolded protein response. Chloramphenicol-treated cells displayed progressive concatemer immobilization with increasing molecular weight and an eight-fold matrix viscosity increase, compatible with increased macromolecular crowding. These results establish that the matrix solvent exhibits macromolecular crowding in functional and dysfunctional mitochondria. Therefore, changes in matrix crowding likely affect matrix biochemical reactions in a manner depending on the molecular weight of the involved crowders and reactants. This makes macromolecular crowding a potential target for manipulation of mitochondrial function in human disease.

Bulthuis, E.P., Dieteren, C.E.J., Bergmans, J., Berkhout, J., Wagenaars, J.A., van de Westerlo, E.M.A., Podhumljak E., Hink, M.A., Hesp, L.F.B., Rosa, H.S., Malik, A.N., Kea-te Lindert, M., Willems, P.H.G.M., Gardeniers, H.J.G.E., den Otter, W.K., Adjobo-Hermans, M.J.W., Koopman, W.J.H. (2023) Stress-dependent macromolecular crowding in the mitochondrial matrix. EMBO J. (in press).

Bulthuis, E.P., Adjobo-Hermans, M.J.W., Willems, P.H.G.M., Koopman, W.J.H. (2019) Mitochondrial morphofunction in mammalian cells. Antioxid. Redox Signal. 30:2066–2109.

Dieteren, C.E.J., Gielen, S.C., Nijtmans, L.G., Smeitink, J.A.M., Swarts, H.G., Brock, R., Willems, P.H.G.M., Koopman, W.J.H. (2011) Solute diffusion is hindered in the mitochondrial matrix. Proc. Natl. Acad Sci. USA 108:8657–8662.

57ASM – 067 | Non-invasive tool for mitochondrial diseases diagnostics

Z. Korandová[1,2]; E. Koňaříková[1]; P. Pecina[1]; A. Pecinová[1]; J. Houštěk[1]; H. Hansíková[2]; T. Honzík[2]; T. Mracek[1]

[1]Laboratory of Bioenergetics, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, Prague, Czech Republic; [2]1st Faculty of medicine, Charles University, Prague, Czech Republic, Prague, Czech Republic

Introduction: Mitochondrial diseases are severe inherited metabolic disorders affecting the paediatric population. They are caused by defects in mitochondrial biogenesis or by mutations in the structural subunits of the oxidative phosphorylation apparatus (OXPHOS). Since mutations in mtDNA are responsible for only 25% of mitochondrial disorders and variant interpretation of next-generation sequencing data benefits from good patient characterization, biochemical analysis still represents a powerful companion tool in diagnostics. Instead of invasive sample collection such as muscle or skin biopsy to derivate a fibroblast culture, we can utilize peripheral blood lymphocytes to identify and characterize the nature of mitochondrial defects.

Materials and Methods: We adapted and optimized highly sensitive oxygraphy of cryopreserved digitonin-permeabilized peripheral blood mononuclear cells to analyse the function of the mitochondrial respiratory chain.

Peripheral blood samples were obtained from approximately 50 patients aged 3 months to 60 years. Out of them, 8 with confirmed MELAS syndrome and 3 with NARP syndrome, and patients with known mutations (SURF1, mt-atp6, AIFM1, LARP, C19orf12) were analysed as well as suspected mitochondrial patients.

Results: Based on our results, we proposed the best respiratory parameters and their ratios predictive of OXPHOS defects. Respiratory rates of coupled and uncoupled respiration, uncoupling control ratio (UCR), and relative respiratory contribution of complex I, II, and GDPH could indicate different OXPHOS defects.

Conclusion: Our study demonstrates sensitive, fast, and non-invasive approach for diagnosing different types of mitochondrial disorders, especially of nuclear genetic origin, manifesting in paediatric patients.

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57ASM – 068 | The role of SURF1 protein in cytochrome c oxidase biogenesis

M. J. Saucedo Rodriguez[1]; P. Pecina[1]; K. Čunátová[1]; M. Vrbacký[1]; A. Pecinová[1]; R. Sobotka[2]; C. Viscomi[3]; M. Zeviani[4]; T. Mracek[1]; J. Houštěk[1]

[1]Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic; [2]Institute of Microbiology, Czech Academy of Sciences, Trebon, Czech Republic; [3]Departement of Biomedical Sciences, University of Padova, Padova, Italy; [4]Department of Neurosciences, University of Padova, Padova, Italy

Introduction: Mitochondria are the main source of cellular ATP, produced in the oxidative phosphorylation apparatus (OXPHOS). One of the crucial components of OXPHOS is cytochrome c oxidase (COX), the terminal enzyme of the respiratory chain that catalyses the transfer of electrons to oxygen. The biogenesis of mammalian COX is a highly regulated process that requires numerous accessory proteins; one of them is SURF1, an assembly factor likely involved in heme insertion or stabilization. Absence of SURF1 in patients results in decreased amount of COX holoenzyme and thus reduced activity. SURF1 defect clinically presents as Leigh syndrome, 30% of Leigh syndrome cases can be accounted to SURF1 mutations.

Results: We analysed patients' fibroblasts and found functional alterations of COX enzyme assembled in the absence of SURF1, including higher electron-transport activity, attenuated proton-pumping and decreased affinity to oxygen. As a next step, we explored COX properties in a mouse SURF1KO model, where we found similar alterations as in human cells. Preliminary analysis of the heme cofactors in COX revealed that SURF1KO muscle mitochondrial contain equal levels of hemes a and b, while controls had almost only heme a. However, due to the differences in COX assembly dynamics between mouse and human, we created a human SURF1KO model in HEK293 cells to study COX hemylation. During the initial characterization of this model, we confirmed affected COX assembly and content. Cellular respiration was affected, which coincides with characteristics that were previously obtained from studies performed in patients' fibroblasts, indicating that we have an accurate model to further study COX biogenesis and metabolic adaptations.

Conclusions: Using a series of SURF1KO models we observed decoupling between electron-transport and proton-pumping activities of COX, which may originate from improper hemylation in the absence of SURF1 protein.

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57ASM – 073 | Role of DAPIT protein in ATP synthase oligomerisation and in regulation of glucose homeostasis

K. Tauchmannova[1]; P. Pecina[1]; A. Pecinova[1]; L. Alan[1]; M. Rodinova[2]; V. Kaplanova[1]; F. Kolar[1]; F. Papousek[1]; D. Habart[3]; L. Kazdova[3]; K. Bardova[1]; M. Pravenec[1]; J. Houstek[1]; T. Mracek[1]

[1]Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic; [2]Department of Paediatrics and Adolescent Medicine, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic; [3]Institute of Clinical and Experimental Medicine, Prague, Czech Republic

Introduction: F_1Fo-ATP synthase is the key enzyme of mitochondrial energy provision, responsible for production of most of the cellular ATP. DAPIT protein (Diabetes Associated Protein in Insulin sensitive Tissues) is a small peripheral subunit implicated in human pathologies but without clear biological function. To study its role, we produced rat knockout model of DAPIT deficiency on unique SHR background.

Results: $DAPIT^{-/-}$ animals were viable and fully assembled ATP synthase was found in normal levels. However, this was predominantly present in the monomeric form. ATP synthase dimers are proposed to play a key role in mitochondrial cristae formation, but we observed only minor changes in cristae morphology in heart of $DAPIT^{-/-}$ animals as well as in HEK293 DAPIT knockdown model. Isolated deficiency of ATP synthase was mild in $DAPIT^{-/-}$ animals, with both ADP phosphorylating and ATP hydrolysing activities reduced by ≈10% in studied tissues.

Body weight of DAPIT$^{-/-}$ animals was reduced by 20%–30% and total adiposity by 40%. We found glucose utilization as preferred source of energy in DAPIT$^{-/-}$ animals. This was replicated at the tissue level, with higher glucose oxidation in DAPIT$^{-/-}$ skeletal muscle. Serum levels of glucose were unchanged, but DAPIT$^{-/-}$ animals were significantly more insulin sensitive with decreased levels of serum insulin as well as area under curve in OGTT test. This is due to the improved peripheral insulin sensitivity, as glucose-stimulated insulin secretion from pancreatic islets was normal in DAPIT$^{-/-}$ animals. High fat diet led to further dissociation of phenotype between control and knockout animals.

Conclusion: Absence of DAPIT protein leads towards preferential oxidation of glucose, increases insulin sensitivity and decreases total adiposity in rat. It implicates that mitochondrial ATP synthase can be directly involved in regulation of glucose homeostasis.

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57ASM – 089 | Miro1 regulates the horizontal transfer of mitochondria in cancer

Z. Nahacka[1]; J. Novak[1,2]; J. Levoux[3]; G. Lopes Oliveira[4]; V. Puttrich[1]; L. Grycova[1]; R. Zobalova[1]; S. Magalhaes-Novais[1]; Z. Lansky[1]; A.-M. Rodriguez[3]; J. Rohlena[1]; J. Neuzil[1,2,5]

[1]Institute of Biotechnology, Czech Academy of Sciences, Prague, Czech Republic; [2]Faculty of Science, Charles University in Prague, Prague, Czech Republic; [3]Université Paris-Est Créteil, INSERM, IB, Créteil, France; [4]Institute for Interdisciplinary Research and Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [5]School of Pharmacy and Medical Science, Griffith University, Southport, QLD 4222, Australia

Introduction: In the absence of functional respiration, mtDNA-devoid cancer cells (ρ0 cells) compensate for this disadvantage by transferring whole mitochondria from surrounding tumour stroma, which allows for development of tumours. The transport and distribution of mitochondria within the cells are dominantly coordinated by Miro1, which together with adaptors TRAK1/2 or Myo19 and molecular motors, facilitates the movement of mitochondria along actin and tubulin cytoskeleton. We hypothesize that Miro1 likewise regulates horizontal transfer of mitochondria between stromal and cancer cells.

Aims:

- To examine the role of Miro1 in horizontal transfer of mitochondria in vitro and in vivo.
- To reveal the molecular mechanism of horizontal transfer of mitochondria and its functional consequences using Miro1 knock-out mice.

Methods: Control and knock-out mice were subcutaneously injected with B16 and B16 ρ0 cells and tumour volume was assessed. Detection of GFP/mKate2 positive cells was assessed by fluorescent cytometry. Movement, attachment and morphology of mitochondria were examined using high-end confocal microscopy. Amount of mtDNA was detected by qPCR and respiration by Oxygraph-2k respirometer. Statistics were done using ANOVA ($p < 0.05$).

Results: We found out that B16 ρ0-derived tumours show delayed formation in Miro1 knock-out mice in contrast to control mice. Using model of Miro1-deficient mice expressing mito::mKate2 (far-red fluorescent protein targeted to mitochondria), we showed decreased frequency of mitochondrial transfer in knock-out animals compared to controls. These results are reflected in the level of mtDNA and basal respiration of tumour cells derived from control and Miro1 knock-out mice. In vitro reconstituted system revealed that lack of Miro1 decreased mitochondria-microtubules attachment, resulting in perinuclear clustering of mitochondria with reduction of mitochondrial movement within and between the cells.

Conclusion: Using Miro1 knock-out mice, we show that tubulin transport of mitochondria regulates their horizontal transfer between cancer and stromal cells.

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57ASM – 092 | Metabolic rewiring in a cellular model lacking ATP synthase

G. Puertas-Frias[1,2]; K. Čunátová[1]; P. Pecina[1]; M. Vrbacký[1]; T. Čajka[3]; J. Houštěk[1]; T. áček[1]; A. Pecinová[1]

[1]Laboratory of Bioenergetics, Institute Of Physiology Of The Czech Acad. Sci., Prague, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Laboratory of Translational Metabolomics, Institute Of Physiology Of The Czech Acad. Sci., Prague, Czech Republic

Introduction: Mitochondria play an essential role maintaining energetic homeostasis and providing metabolic intermediates for anabolic pathways. They conduct oxidative phosphorylation (OXPHOS) through the action of the five complexes, which couple the redox reactions in the matrix (i.e. TCA cycle) to the electron transport chain (complexes I to IV) and ATP synthesis (complex V or ATP synthase). Defects in OXPHOS can impair cellular metabolism,

leading to human disease, and trigger the rearrangement of metabolic pathways to safeguard homeostasis.

Results: In this study, we assess metabolic rewiring in HEK293 cells with β subunit knock out modelling ATP synthase deficiency. This led to a total loss of the assembled enzyme and secondary downregulation of respiratory chain complexes. As a result, coupled respiration was impaired and uncoupled respiration was largely suppressed, while glycolytic rate was elevated to support ATP production. Through stable isotope tracing of U13C labelled glucose and glutamine, we observed that glucose-derived pyruvate is mostly converted to lactate to maintain the high glycolytic rate and does not enter TCA cycle. Reductive carboxylation of glutamine was in turn responsible for producing citrate and the terminal TCA metabolite pools of malate and fumarate. Surprisingly, the NAD+/NADH ratio was unchanged despite the profound impairment on mitochondrial oxidative capacity, indicating that this rearrangement of the TCA cycle acts as an adaptive mechanism to maintain cellular redox balance.

Conclusions: Our results show how metabolic rewiring triggered by ATP synthase deficiency serve as an adaptive mechanism to maintain energetic and redox homeostasis in cultured human cells.

The project is supported by the Grant Agency of the Czech Republic (21-18993S).

57ASM – 098 | Mitochondrial Uncoupling Proteins (UCPs) are key modulators of Leydig cells' mitochondrial activity

B. C. Matos[1,2]; B. Guerra-Carvalho[1,2,3,4,5]; D. F. Carrageta[1,2,3]; R. L. Bernardino[1,3]; P. F. Oliveira[4]; M. G. Alves[1,2,3]

[1]Clinical and Experimental Endocrinology, UMIB – Unit for Multidisciplinary Research in Biomedicine, ICBAS -School of Medicine and Biomedical Sciences, University Of Porto, Porto, Portugal; [2]Laboratory of Physiology, Department of Imuno-physiology & pharmacology, School of Medicine and Biomedical Sciences (ICBAS), University Of Porto, Porto, Portugal; [3]Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, Porto, Portugal; [4]LAQV-REQUIMTE and Department of Chemsitry, Universidade de Aveiro, Aveiro, Portugal; [5]Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal

Background: Hypogonadism is a common comorbidity of metabolic diseases, although the mechanisms mediating the decreased Leydig cells' testosterone production remain poorly understood. Mitochondrial uncoupling proteins (UCPs) are essential regulators of cellular metabolism, mitochondrial activity, and reactive oxygen species (ROS) production. Notably, the expression of UCPs in Leydig cells and their role in these cells' mitochondrial activity and steroidogenesis remains unknown. Herein, we aimed to identify the expression of UCP1, UCP2, and UCP3 in mouse Leydig cells (mLCs) and evaluate their function in mitochondrial activity (by using genipin, a selective UCP inhibitor).

Materials and Methods: Cultures of mLCs (BLTK-1 cell line) were used ($n = 10$). Total RNA was extracted, and Ucp1-3 NA expression was determined by RT-PCR. UCP1-3 protein expression was detected by Western Blot and immunofluorescence. UCPs were inhibited by incubation with genipin (0.5, 5, 50, 100 μM). After 24 h, cellular proliferation and viability were assessed. Mitochondrial activity was assessed by the Seahorse XF Cell Mito Stress assay. Data were tested with repeated measures one-way ANOVA with post hoc Dunnett's multiple comparisons tests ($p < 0.05$ was considered significantly different).

Results: We were able to identify the expression of UCP1-3 in mLCs. The inhibition of UCPs led to a dose-dependent decrease in cell proliferation and viability. A dose-dependent effect was also observed on the mitochondrial activity of Leydig cells, whose respiratory capacity was severely impaired after the inhibition of UCPs.

Conclusion: This work demonstrated, for the first time, that Leydig cells express UCP1, UCP2, and UCP3. UCPs inhibition compromises mLCs mitochondrial activity, suggesting a potential role in steroidogenesis. These results highlight a possible direct role of UCPs in the crosstalk between metabolic diseases, mitochondrial dysfunction, steroidogenesis, and hypogonadism.

57ASM – 103 | Impaired mitochondrial and metabolic function of fibroblasts derived from patients with Neurodegeneration with Brain Iron Accumulation

Wydrych[1]; B. Pakuła[1]; P. Jakubek[1]; J. Janikiewicz[1]; A. M. Dobosz[1]; A. Cudna[2]; A. Antos[2]; M. Rydzewski[2]; M. Cwyl[3,4]; M. Popielarz[4]; M. Skowrońska[2]; I. Kurkowska-Jastrzębska[2]; A. Dobrzyń[1]; M. Lebiedzińska-Arciszewska[1]; M. R. Wieckowski[1]

[1]Nencki Institute Of Experimental Biology Pas, Warsaw, Poland; [2]Institute of Psychiatry and Neurology, Warsaw, Poland; [3]Association NBIA Poland, Warsaw, Poland; [4]Warsaw University of Technology, Warsaw, Poland

Introduction: Neurodegeneration with Brain Iron Accumulation (NBIA) is a rare disease characterized by the deposition of the iron in the brain. One of the

NBIA subtypes is mitochondrial membrane protein-associated neurodegeneration (MPAN) caused by mutation in C19orf12 gene, but the molecular mechanism underlying the disease is still not understood. The goal of our research is complex characterization of the fibroblasts derived from MPAN patients to determine metabolic and oxidative-stress related factors, to identify affected metabolic pathways associated with the clinical phenotype.

Materials and Methods: Fibroblast obtained in a cohort of 11 MPAN patients and 4 healthy donors. Fibroblasts were cultured in standard or OXPHOS supporting conditions. OXPHOS function was evaluated using Clark-type electrode. Metabolic activity and redox status were examined by fluorescent detection methods. The level of individual proteins was examined by western blot. Correlations that could distinguish controls from NBIA patients were determined by PCA.

Results: Fibroblasts derived from MPAN patients have exhibited affected metabolic and mitochondrial activity compared to control fibroblasts. In MPAN fibroblasts decreased mitochondrial respiratory chain activity has been found. Under conditions favouring mitochondrial metabolism, patients' fibroblasts exhibited slower proliferation without any signs of increased cell death. Moreover, under these conditions alterations in basal, maximal and ATP related oxygen consumption rates as well as alterations in ROS levels and antioxidant defence status become more visible in MPAN patients' fibroblasts. PCA analysis showed a clear separation of MPAN patients' and controls' metabolic profiles at OXPHOS supporting conditions.

Conclusions: Manifestation of impaired mitochondrial and metabolic function of MPAN patients' fibroblasts is better visible at OXPHOS supporting conditions.

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57ASM – 104 | Role of Miro1 in mitochondrial mobility between cancer and stromal cells

J. Novak[1,2]; Z. Nahacka[1]; J. Levoux[3]; G. Lopes Oliveira[1,4]; V. Puttrich[1]; L. Grycova[1]; R. Zobalova[1]; S. Magalhaes-Novais[1]; Z. Lansky[1]; A.-M. Rodriguez[3]; J. Rohlena[1]; J. Neuzil[1,2,5]

[1]Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic; [2]Charles University in Prague, Faculty of Science, Prague, Czech Republic; [3]Université Paris-Est Créteil, INSERM, IB, Créteil, France; [4]Institute for Interdisciplinary Research and Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [5]School of Pharmacy and Medical Sciences, Griffith University, Southport, Australia

Introduction: Cancer cells require mitochondria to provide energy and building blocks for tumour growth. They compensate for mitochondrial damage resulting from therapy or mutational burden by ‘kidnapping’ healthy organelles from surrounding tissues. Mitochondria are ‘hijacked’ by several mechanisms, mostly by means of transport via thin protrusions called tunnelling nanotubes (TNTs). Several publications and our in vivo experiments point to the important, yet enigmatic, role of the small GTPase Miro1 in this process.

Aim: To elucidate the role of Miro1 in mitochondrial transfer from mesenchymal stromal cells (MSCs) to cancer cells with mitochondrial damage.

Methods: We have used three levels of experimental complexity. First, a simplified cell-free system of reconstituted mitochondria, molecular motors and tubulin that allows us to track the movement of a single organelle along microtubules. Second, in vitro, we utilized patterned coverslips, live cell imaging and mono- or co-cultures of MSCs and cancer cells to quantify the morphology and dynamics of the mitochondrial network. Third, we used freshly isolated cells from mouse tumours to confirm transfer of mitochondria in vivo.

Results: The lack of Miro1 reduced the number of mitochondria associated with and moving along microtubules. The absence of Miro1 resulted in decreased mobility and perinuclear localization of mitochondria in cancer cells and MSCs in vitro. Similarly, the number of mitochondria in TNTs between MSCs was reduced. Finally, we identified mitochondrial transfer via TNTs connecting cancer cells and MSCs in vitro, and we isolated cancer cells with acquired mitochondria directly from mouse tumours.

Conclusion: Our results illustrate the importance of mitochondrial transfer in the context of cancer. In particular, we have elucidated the role of Miro1 in this process, supporting the model where it recruits mitochondria to microtubules, secures their movement towards the cell

periphery and inside the TNTs, and ultimately into the recipient cancer cell.

57ASM – 105 | Hepatic manifestation of oxidative stress in patients with non-alcoholic fatty liver disease

P. Jakubek[1]; P. Kalinowski[2]; A. Karkucinska -Wieckowska[3]; A. Kaikini[1]; B. Kruk[4]; M. Lebiedzińska-Arciszewska[1]; W. Grajkowska[3]; P. Pinton[5]; P. Milkiewicz[2]; M. Grąt[2]; M. Pronicki[3]; M. Krawczyk[4,6]; M. R. Wieckowski[1]

[1]Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2]Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland; [3]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland; [4]Laboratory of Metabolic Liver Diseases, Department of General, Transplant and Liver Surgery, Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland; [5]Department of Medical Sciences, Section of Experimental Medicine, Laboratory for Technologies of Advanced Therapies, University of Ferrara, Ferrara, Italy; [6]Department of Medicine II, Saarland University Medical Center, Saarland University, Homburg, Germany

Introduction: Non-alcoholic fatty liver disease (NAFLD) affects around 30% of the global population and as such, it has become the most common liver disease worldwide. Despite its benignity in most cases, the disease can progress to non-alcoholic steatohepatitis (NASH) in approximately 20% of NAFLD patients. The mechanisms that drive disease progression are not yet fully understood, however, oxidative stress is proposed as one of the key factors involved in this process. This study aimed to examine whether the disturbed redox status observed in preclinical models of NAFLD is also present in NAFLD patients.

Materials and Methods: Liver samples were obtained intraoperatively in a cohort of 32 obese patients scheduled for bariatric surgery (14 males, 18 females, age range 43.5 ± 9.6 years). Biopsies from 6 patients without NAFLD served as control. NAFLD staging was performed histopathologically. The parameters assessing hepatic redox status were examined by western blot. Correlations that could distinguish controls from NAFLD patients or NASH patients from those without NASH were determined by PCA.

Results: The level of protein carbonylation was significantly lower in liver samples obtained from NAFLD patients compared to controls. The levels of thioredoxin and mitochondrial superoxide dismutase were significantly decreased in NASH patients. Regardless of the stage of NAFLD, a downward trend towards decreased antioxidant status was shown by lower levels of cytosolic superoxide dismutase, thioredoxin reductase 1, peroxiredoxin 1, and increased level of 4-hydroxynonenal, which is a marker of lipid peroxidation. PCA analysis showed a clear distinction between NAFLD patients and controls, however, it failed to differentiate patients with and without NASH.

Conclusions: Manifestation of oxidative stress can be already observed in the hepatic tissue of patients without NASH, with a few of the parameters indicating oxidative stress aggravation along NAFLD progression.

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57ASM – 106 | Horizontal transfer of mitochondria restores respiration before formation of brain tumours

P. Brisudová[1,2]; R. Zobalová[1]; Š. Dvořáková[1]; Z. Nahácka[1]; J. Novák[1,2]; J. Neužil[1,2,3,4]

[1]Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Prague-West, Czech Republic; [2]Faculty of Science, Prague, Czech Republic; [3]First Faculty of Medicine, Prague, Czech Republic; [4]School of Pharmacy and Medical Science, Qld, Australia

Introduction: Horizontal transfer of mitochondria has been shown to take place between mammalian cells in vitro and in vivo in various experimental set-ups. This form of mitochondrial function restoration was described in different physiological and pathological conditions such as wound healing or neurodegenerative diseases, but there is a particular interest in the movement of mitochondria in the area of cancer biology. Our laboratory has shown that transport of healthy mitochondria from tumour-surrounding cells to the neoplastic cells can support proliferation and increase tumour-forming capacity. This was demonstrated in vivo in subcutaneous tumours derived from melanoma and breast cancer cells with dysfunctional respiration (Bajzikova et al., 2019). However, little data were available about the role of mitochondria acquisition by brain tumours.

Materials and Methods: To investigate the possibility of mitochondrial transfer in brain tumours we employed orthotopic mouse models of glioblastoma multiforme. We grafted 5×10^4 cells of glioblastoma cells GL261 into the caudate putamen of C57BL/6 mice. Two types of GL261 cells were injected, either parental cells with intact mitochondrial DNA (mtDNA) or rho0 cells devoid of mtDNA.

Results: We observed a considerable delay in the formation of tumours from rho0 cells compared to the parental cells. The delay is most likely caused by the necessity for mitochondrial acquisition by rho0 cells to meet the

bioenergetic/anabolic needs for tumour proliferation. By sequencing of mtDNA, we showed that cells without mtDNA acquire mitochondria from the host after engraftment into the brain. Moreover, these cells showed restored mitochondrial cristae structure, mtDNA level, transcription of mtDNA-coded genes and formation of respiratory supercomplexes resulting in mitochondrial respiration recovery and accompanying lower levels of glycolysis.

Conclusion: Our findings suggest that horizontal transfer of mitochondria occurs in glioblastoma in vivo and that recovery of respiration is required for brain tumour formation.

57ASM – 123 | Determination of the effect of melatonin and everolimus co-administration on cell viability and mitochondrial respiration in MCF-7 cells

S. Demirkesen; C. Aral; Y. İriağaç; E. Selçuk Şeber
Namik Kemal University, Tekirdag, Turkey

Introduction: The most common type of breast cancer in women is hormone receptor-positive (HR +) and human epidermal growth factor-2 (HER-2-) negative. Despite the response to hormonal therapy in treating this type of breast cancer, resistance mechanisms may occur over time. One of the most critical resistance mechanisms known here is the activation of the mTOR pathway. Therefore, suppressing mTOR activity and minimizing the side effects are important for understanding and developing anti-tumour activity.

Aim: This study set out to evaluate the effect of the combination of everolimus and melatonin on cell death in MCF-7 cells and to determine whether there is a difference in terms of mitochondrial respiration.

Method: In the scope of the research, IC50 doses were determined by MTT test, changes in cell number in cell cycle stages and the presence of apoptotic cell death were determined by flow analysis. In order to determine the mitochondrial respiration rate, the respiratory rate over complex-II was calculated and membrane potential was determined by fluorescence microscopy with JC-1 staining. The evaluation of mTOR pathway protein expressions and p62 and LC-3 protein expressions for autophagic cell-death were examined by immunoblotting, and the amount of LC-3 was also evaluated by fluorescence microscopy for autophagic activation.

Results: As a result, it was determined that cell viability decreased, apoptotic cell death increased, autophagic activation occurred and an improvement in mitochondrial respiration rate occurred through complex II in MCF-7 cells as a result of the combined application of melatonin and everolimus. Melatonin plays a role in increasing apoptotic and autophagic effects as a result of co-administration compared to everolimus alone. In addition, supporting the improvement in mitochondrial respiration indicates a high potential for future studies to increase the antitumour effect. This study was supported by TNKU-BAP (01.22.355) and TÜBİTAK 1002 (222S127).

57ASM – 127 | The role of complex II aberrations in metabolism and susceptibility to mitochondria-targeted treatment of renal cancer

S. Bálintová[1,2]; Š. Dvořáková[1]; K. Kološtová[3]; V. Bobek[3,4,5,6]; J. Neužil[1,7]
[1]Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Laboratory of Personalized Medicine, Oncology Clinic, University Hospital Kralovske Vinohrady, Prague, Czech Republic; [4]3rd Department of Surgery University Hospital Motol and 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [5]Department of Thoracic Surgery, Masaryk's Hospital, Krajska Zdravotni a.s., Usti nad Labem, Czech Republic; [6]Department of Thoracic Surgery, Lower Silesian Oncology, Pulmology and Haematology Center and Medical University Wroclaw, Wroclaw, Poland; [7]School of Pharmacy and Medical Science, Griffith University, Southport, Australia

Introduction: Mitochondria represent a plausible target for cancer therapy. We developed a mitochondrially-targeted analogue of tamoxifen, MitoTam, acting via targeting mitochondrial respiratory complex I, which successfully passed Phase I/Ib clinical trial and was found to be the most potent in patients with renal cell carcinoma. Clinical evidence shows that mitochondrial respiratory complex II abnormalities frequently manifest in renal cell carcinoma patients and are linked to their lower survival. It was also shown in SDHB-deficient renal cancer that aberrations in complex II seem to have an impact on complex I status in the tumour cells.

Aims: The proposed work aims to decipher the influence of renal tumour aberrations, related to tumour metabolism, on the effectiveness of MitoTam treatment.

Methods: We used murine renal adenocarcinoma cell line RenCa and its derived SDHB knock-out cells, BALB/c mice with subcutaneously injected RenCa cells, and kidney and tumour tissues from renal cell carcinoma patients.

Results: We found suppressed levels of complex II subunits and assembly factors, complex II- and complex I-dependent respiration, and complex II-linked succinate: quinone reductase activity in tumour samples from some renal cell carcinoma patients. Knocking out SDHB in RenCa cells led to decreased mitochondrial respiration, complex II

assembly and enzymatic activity, expression of subunits and assembly factors of complex II, and moreover, diminished supercomplexes containing complex I. Subcutaneous tumours formed in mice from SDHB knock-out RenCa cells failed to respond to treatment with MitoTam.

Conclusions: Based on our data, it appears that there are renal cell carcinoma tumours with profound metabolic disturbances. How these aberrations interfere with the therapeutic effect of MitoTam, therefore, holds great clinical importance.

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57ASM – 129 | Deficiency of Miro1 attenuates the migratory properties and metastatic potential of cancer cells

G. Oliveira[1,2,3]; J. Smigova[4]; J. Novak[1,4]; S. Stemberkova-Hubackova[1,5]; J. Brabek[4]; P. Oliveira[2,3]; Z. Nahacka[1]; J. Neuzil[1,4,6]

[1]Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic; [2]Center for Neuroscience and Cell Biology, Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [3]Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; [4]Faculty of Science, Charles University, Prague, Czech Republic; [5]Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [6]School of Pharmacy and Medical Science, Griffith University, Southport, Qld, Australia

Introduction: Miro1 is a Rho GTPase and a key regulator of mitochondrial trafficking, localized in the outer mitochondrial membrane. Cell migration is an energy-consuming process, hence the mitochondrial position within cells can have an impact on the motility of cells. Cancer cell migration is crucial during invasion, being the initial step of metastasis. Here we aimed to address whether Miro1 regulates cell invasion and the formation of metastases.

Methods: Deletion of Miro1 in B16 melanoma cells was generated by CRISPR-Cas12a. The following methods were used: mitochondrial membrane potential/reactive oxygen species/mitochondrial mass (flow cytometry), ATP (luminescent assay), mitochondrial respiration (Oxygraph-2k respirometer), and adhesion (confocal microscopy). Scratch assay ('wound healing') and 3D-cell culture were performed for cell migration/invasion analysis. C57BL/6J mice were injected intravenously with B16 and B16 Miro1-deficient cells, and lungs were examined for the presence of metastatic tumours by H&E staining.

Results: Miro1-deficient B16 cells showed a decrease of approximately 20% in ATP total levels when compared to control, but no difference was found in mitochondrial mass, mitochondrial membrane potential, or respiration. We found that deficiency of Miro-1 decreased the wound healing area in Miro1-deficient cells (50%), as well as the invasion index (60%), and the outward migration speed of cells from spheroids (45%). Furthermore, we observed reduced adhesions in Miro1-deficient cells. Finally, in vivo data showed that mice injected with Miro1-deficient cells formed fewer metastatic foci in the lungs when compared to control cells.

Conclusions: Our data show that Miro1 regulates the migratory and metastatic capacity of B16 cancer cells via the positioning of mitochondria and coordinating the actin cytoskeleton, which suggests that targeting of Miro-1 could be a potential therapeutical approach.

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57ASM – 132 | The Effect of LifeLong Exercise on the Mitochondrial Respiratory Chain Function in Human Peripheral Blood Mononuclear Cells

A. Pedrosa[1,2]; C. Soares[1,3]; S. Pinho[2,4]; P. Sousa-e-Silva[1]; P. Filipe[5]; T. Azul[5]; P. J. Oliveira[2]; A. I. Duarte[2]; L. Rama[1]; A. M. Teixeira[1]; V. A. Sardão[2,6]

[1]University of Coimbra – Research Unit for Sport and Physical Activity (CIDAF, UID/PTD/04213/2020), Faculty of Sport Sciences and Physical Education (FCDEF-UC), Portugal;, Coimbra, Portugal; [2]CNC – Center for Neuroscience and Cell Biology, CIBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra; 3004–504 Coimbra, Portugal, Coimbra, Portugal; [3]Molecular Physical Chemistry R&D Unit Coimbra, Portugal, Coimbra, Coimbra; [4]PDBEB – PhD Programme in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal, Coimbra, Coimbra; [5]Centro Hospitalar Universitário de Coimbra, Coimbra, Portugal; [6]Multidisciplinary Institute of Aging (MIA Portugal), University of Coimbra; 3004–504 Coimbra, Portugal, Coimbra, Portugal

Introduction: Regular physical exercise improves health and life quality in elderly population [1]. It is also associated with alterations in peripheral blood mononuclear cells (PBMC) composition, enhancing the function of the immune system. PBMC are also a powerful tool for research and clinical applications, frequently used to study several aspects of cellular biology and pathology. This

study aimed to perform a real-time cellular metabolic analysis to understand the effect of lifelong training (LLT) on PBMC metabolism and mitochondrial performance.

Method: Two groups, comprising 9 male masters athletes (MA, aged 52.77 ± 7.03 years) and 6 sedentary men (SG, aged 51 ± 6.78 years) performed an incremental test until exhaustion on a cycle ergometer. Blood samples were collected before (Pre) and after the exercise protocol (Post). Measurements of oxygen consumption rate (OCR) and proton efflux rate (PER) were performed on isolated PBMCs, by using the Extracellular flux XFe96 Analyser. Mitochondrial respiratory complexes subunits were quantified by Western Blot.

Results: Obtained results showed an increase in basal, ATP-linked, non-mitochondrial OCR and proton leak-associated OCR in PBMC isolated from MA when compared with SG group. The protein levels of NDUFB8, SDHB, UQCRC2 and COXI were increased in both groups upon the cycle ergometer incremental test to exhaustion.

Conclusions: Our results suggest that LLT increases mitochondrial function and may attenuate the age-related decline in mitochondrial function, in PBMC.

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Keywords: "Masters Athletics"; "Aging"; "Mitochondria"; "Lifelong Exercise".

Reference:

1. Granata, C., Caruana, N.J., Botella, J. et al. High-intensity training induces non-stoichiometric changes in the mitochondrial proteome of human skeletal muscle without reorganization of respiratory chain content. Nat Commun 12, 7056 (2021). https://doi.org/10.1038/s41467-021-27153-3

57ASM – 144 | Modulation of mitochondrial respiration by pro-apoptotic proteins Bax and Bak

D. Sovilj[1,2]; C. Kelemen[1]; V. Filiomenko[3]; H. Raabova[3]; M. Anderova[4]; J. Kriska[4]; Z. Hermanova[4]; J. Neuzil[1,5]; L. Andera[1,3]

[1]Institute of Biotechnology, Vestec, Czech Republic; [2] Faculty of Science, Charles University, Prague, Czech Republic; [3]Institute of Molecular Genetics, Prague, Czech Republic; [4]Institute of Experimental Medicine, Prague, Czech Republic; [5]School of Pharmacy and Medical Sciences, Griffith University, Southport, Australia

Introduction: Besides their well-known role in apoptosis, Bcl-2 family proteins are involved in cell death-unrelated processes such as modulation of Ca2+ homeostasis, mitophagy, DNA damage response, etc. Several studies also linked Bcl-2 family proteins to cellular metabolism affecting it in both positive and negative ways; however, very little is known about mechanisms behind this.

Aims: Using cells with eliminated expression of the pro-apoptotic Bax and Bak proteins, we aimed at dissecting their role in mitochondrial metabolism and at uncovering relevant signalling pathways.

Methods: The CRISPR/Cas9 technique was used for the elimination of expression of pro-apoptotic proteins Bax and Bak in cancer cell lines of different origin. Mitochondrial respiration and glycolysis was determined using the Seahorse XFe95 and Oxygraph2k analyzers assay, levels of selected metabolites were assessed by LC–MS analysis. Growth profiles were evaluated by the Incucyte SX1 instrument.

Results: This study shows that two main pro-apoptotic proteins Bax and Bak modulate cancer cell respiration in a cell type-dependent manner. Glioblastoma U87-MG Bax/Bak deficient cells showed increased mitochondrial respiration compared to parental cells. Increased respiration was accompanied by faster proliferation, increased MMP, and metabolic changes as increased levels of NADH and aspartate. Respiration was increased by means of higher activity of the respiration complex I (RCI) likely via increased expression of RCI proteins. The mitochondrial subunit of RCI mtND5 was found upregulated on both transcriptional and protein level, apparently as a consequence of higher expression of TEFM, a regulator of mitochondrial genome transcription and NA processing. In contrast to glioblastoma, colorectal HCT-116 showed no differences in respiration upon Bax/Bak elimination, while B-cell lymphoma cell lines HBL-2, Upf1-G, and Upf1-H showed decreased respiration.

Conclusion: Our study documents that Bax and Bak can modulate mitochondrial respiration independently of their apoptotic function. This effect of Bax/Bak on mitochondrial respiration is likely cell-type specific.

57ASM – 150 | Modulatory effect of melatonin on the mitochondrial permeability transition pore

A. Michalowski[1]; A. Szymanska[2]; P. Pinton[3]; S. Rodziewicz-Motowidlo[2]; M. R. Wieckowski[1]

[1]Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2]Department of Biomedical Chemistry, University of Gdansk, Gdansk, Poland; [3]Department of Medical Sciences, Section of Experimental Medicine, Laboratory for Technologies of Advanced Therapies, University of Ferrara, Ferrara, Italy

Introduction: In the last decade, several publications indicated a link between the progression of Non-Alcoholic

Fatty Liver Disease and mitochondrial dysfunction. One of said dysfunctions involves opening of the mitochondrial Permeability Transition Pore (mPTP). Although the mPTP functionality is known, still there is no consensus regarding its exact structure. The aim of our study was to investigate whether melatonin, a hormone with antioxidant properties, could have modulatory effect on the mPTP and thus alleviate mitochondrial dysfunction observed during NAFLD development and progression.

Materials and Methods: Effect of melatonin on mitochondrial function and mPTP functionality has been tested on isolated mice liver mitochondria. Interestingly, C57BL/6J mice used by us have stable melatonin level regardless to circadian clock. Fluorometric, spectrophotometric and oximetric techniques tests have been performed to investigate modulatory effect of melatonin on the mPTP sensitivity to calcium, mitochondrial respiratory chain, focusing on ATPase/synthase activity of mitochondrial ATP synthase.

Results: We have found that 100 μM melatonin shows modulatory effect on the mPTP when induced by calcium. Interestingly, in range of 10 nM – 100 μM melatonin does not have any significant effect on mitochondrial basal and maximal rate of oxygen consumption as well as on the mitochondrial membrane potential. This suggests that melatonin-mediated mPTP pore opening regulation is not related to the melatonin effect on mitochondrial bioenergetics. Moreover, we did not observe any effect of melatonin on ATPase/synthase activity of mitochondrial ATP synthase.

Conclusion: Based on the results obtained so far, melatonin is able to modulate the mPTP activity and, simultaneously, do not interfere with the mitochondrial bioenergetic parameters. Interestingly, mPTP melatonin-mediated effect is not related to ATPase/synthase activity of mitochondrial ATP synthase considered to form mPTP. Hence, it can be further investigated as a potential drug compound that might alleviate mitochondrial dysfunction observed during NAFLD development and progression.

57ASM – 151 | Simultaneous targeting of mitochondrial metabolism and immune checkpoints shows a new strategy for renal cancer therapy

R. Zobalova[1]; S. Stemberkova-Hubackova[1]; M. Dubisova[1,2]; J. Smigova[2]; Z. Bielcikova[3]; J. Brabek[2]; J. Stursa[1]; L. Werner[1]; J. Neuzil[1,2,4]

[1]Institute of Biotechnology, Czech Academy of Sciences, Vestec, Prague-West, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]General University Hospital, Prague, Czech Republic; [4]School of Pharmacy and Medical Science, Griffith University, Southport, Australia

Introduction: Mitochondria present an emerging target for cancer treatment. Mitochondrially targeted tamoxifen (MitoTam), a potential anti-cancer agent, has recently undergone phase I/Ib clinical trial in patients with various solid tumours. Patients with clear cell renal carcinoma (RCC) showed the highest response of the tested diagnoses.

Aims: To study the effect of MitoTam on renal cancer in vitro and in vivo to reveal its mechanism of action in more detail and better understand its benefit for patients.

Methods: Using primarily the murine RenCa RCC cells and the derived syngeneic mouse tumour model, we studied mechanism of MitoTam toxicity including the mode of death, the role of mitochondria in the effects of the agent, and its efficacy in suppressing syngeneic tumours in mice alone and in combination with immune checkpoint inhibitors (ICIs) PD-1 and PD-L1.

Results: Our findings show a complex effect of MitoTam on mitochondrial function and integrity of renal cancer cells. The agent inhibits complex I-dependent respiration and lowers mitochondrial potential, which results in increased ROS production and activation of necroptosis as the major mode of cell death. We observed a switch to aerobic glycolysis in MitoTam-treated cells, documented both by metabolomic and bioenergetic analyses. MitoTam reduced growth of renal tumours as well as metastatic spread of tumour cells via its specific targeting of malignant tissue in a mouse tumour model. Moreover, combination of MitoTam with immunotherapy to enhance its anti-cancer efficacy shows significantly increased suppression of tumour growth as well as increased survival of experimental animals compared to single agent treatment.

Conclusion: Our data provide a mechanistic rationale for testing of combinatorial therapy with MitoTam plus ICIs in renal carcinomas in Phase 2 trial.

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57ASM – 152 | Metabolic reprograming in hepatocellular carcinoma cellular model – Involvement of mitochondria – Endoplasmic reticulum contact sites

S. Karimi[1]; S. Missiroli[2]; P. Pinton[2]; M. Wieckowski[1]; M. Lebiedzinska-Arciszewska[1]

[1]Nencki Institute of experimental biology PAS, Warsaw, Poland; [2]Signal Transduction Laboratory Dept. of Medical Sciences, University of Ferrara, Ferrara, Italy

Introduction: The oncogenic process is accompanied by metabolic reprogramming from oxidative phosphorylation governed by mitochondria to aerobic glycolysis to promote enhanced proliferation of cancer cells. This shift is associated with affected signalling regulating levels and/or activities of enzymes involved in mitochondrial metabolism. Many proteins involved in the regulation of metabolic processes are localized permanently or temporarily in mitochondria-endoplasmic reticulum contact sites (MERCs). Since MERCs are crucial in processes such as phospholipid biosynthesis, calcium signalling, apoptosis, and autophagy, we hypothesize MERCs' involvement in cancer cell metabolism regulation. Our studies aim to investigate the range of metabolic reprogramming accompanying the shift from the highly proliferative phenotype of hepatocellular carcinoma (HCC) model – HepG2 cells, to confluent HepG2/C3A which resembles differentiated hepatocytes culture. We investigated whether affected metabolic signalling is related to changes in MERCs' proteomic composition and number.

Materials and Methods: HepG2 and HepG2/C3A cultures. Protein levels were examined by western blot. Metabolic activity was measured by resazurin assay and mitochondrial membrane potential was measured by TM with the use of multi-well plate reader.

Results: Confluent HepG2/C3A culture differs from confluent HepG2 in morphology and growth pattern. The loss of the HepG2/C3A proliferative phenotype was confirmed by a decrease in the HCC marker – alpha-fetoprotein AFP level. The metabolic activity of confluent HepG2/C3A is lower in comparison to highly proliferative HepG2. In HepG2 high metabolic activity was accompanied by increased levels of hexokinase II and OXPHOS subunits, particularly of complexes II and IV, which may suggest metabolic reprogramming. Moreover, we observed differences in the levels of MERCs' proteins such as IP3R-3.

Conclusions: These findings suggest that using confluent HepG2/C3A culture compared to HepG2 may reveal metabolic and proteomic alterations related to MERCs that might be responsible for cancer phenotype.

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57ASM – 158 | Characterization and modulation of MitoTam-induced cell death in breast carcinoma cells

M. Hrysiuk[1,2]; D. Sovilj[1,2]; C. Kelemen[1]; L. Andera[1,3]; J. Neuzil[1,2,4,5]

[1]Institute of Biotechnology of the Czech Academy of Sciences, Prague-West, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic; [4]First Faculty of Medicine, Charles University, Prague, Czech Republic; [5]School of Farmacy and Medical Science, Charles University, Ptague, Czech Republic

Introduction: Recent years brought many breakthrough discoveries in cancer research and therapy, malignant diseases such as breast cancer still remain one of the major health threats worldwide. Cancer cells usually gain resistance to regulated cell death (RCD) and thus there is a constant need for novel anti-cancer drugs and treatment protocols targeting RCD pathways in cancer cells.

Aims: Among the novel RCD-inducing agents belongs also mitochondria-targeted tamoxifen – MitoTam, which is the major focus of this study with the following aims:

1. Analysis of the expression of RCD-related genes in human breast cancer cells and determination of respiration/glycolysis status.
2. Induction and time-lapse profiling of RCD triggered by MitoTam in breast cancer cells and its modulated by metabolic/energy pathways (MEP) inhibitors and BH3 mimetics.

Methods: In this study we employed the XFe96 Seahorse/Agilent analyser (mitochondrial respiration and cellular glycolysis), Oxygraph2k/Oroboros (mitochondrial respiration), western blotting and time-lapse monitoring of cell proliferation and cell death using Incucyte SX1 and Lumascope LS720 instruments.

Results: With few exceptions, all cell types expressed essential RCD-related proteins and responded to MitoTam treatment with various efficacy. The glycolysis-preferring breast cancer cell lines such as MDA-MB-231 are more resistant to MitoTam treatment than mitochondrial respiration-based MDA-MB-453 cells. The co-treatment experiments showed strong enhancing effect of Bcl-XL inhibitor A1155463 on MitoTam-induced RCD in MDA-MB-231 and T47D cells and of Mcl-1 inhibitor S63845 on MDA-MB-453 cells. Selected MEP inhibitors such as the lactate dehydrogenase inhibitor (R)-GNE-140 or pyruvate dehydrogenase kinases inhibitor JX06 greatly enhanced MitoTam-induced RCD of breast cancer cells.

Conclusions:

1. Breast cancer cells with higher levels of mitochondrial respiration appeared to be more sensitive to MitoTam treatment.
2. Breast cancer cells resistant to MitoTam-induced RCD can be sensitized by their co-treatment with (a) BH3 mimetics targeting Bcl-XL or Mcl-1 or (b) selected MEP inhibitors preferentially targeting glycolysis-affecting/modulating signalling.

57ASM – 161 | Dysregulation of mitochondrial bioenergetics leads to elevated ROS levels and promotes programmed developmental progression of Trypanosoma brucei

M. Kunzová[1,2]; E. Dolezelova[1]; A. Zíková[1,2]
[1]*Biology Centre CAS, Institute of Parasitology, České Budějovice, Czech Republic;* [2]*Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*

Background: In the last decade, the importance of mitochondrially derived reactive oxygen species (OS) as signals in intracellular communication has been sealed. These though-to-be harmful molecules influence cellular faith decisions and can drive cells towards proliferation, apoptosis or differentiation. The OS are mainly generated as by-products during the process of oxidative phosphorylation (OXPHOS), which is completed by FoF1-ATP synthase with the formation of ATP. In trypanosomes, unicellular parasites undergoing programmed life cycle development, the cellular differentiation is linked to increased mitochondrial metabolic activity and OS production.
Aims: We aim to investigate the relationship between the mitochondrial bioenergetics, OS production and the parasite differentiation efficiency using genetically engineered trypanosomes that lack or have excess of Inhibitory factor 1 (IF1), a unidirectional inhibitor of FoF1-ATP synthase.
Method: To determine the effects of IF1 ablation or its overexpression on OS production and mitochondrial bioenergetic fitness during parasite differentiation we examined levels of mitochondrial membrane potential, cellular respiration, AMP/ADP/ATP, OXPHOS complexes, and mitochondrial and cellular reactive oxygen species by numerous assays.
Results: We demonstrated that the absence of IF1 leads to dysregulation of mitochondrial bioenergetics associated with increased proline-dependent respiration by alternative oxidase, elevated OS and earlier activation of AMPK kinase which promotes life cycle transition to the quiescent metacyclic form. In contrast, overexpression of IF1 results in lower levels of ROS and arrested differentiation of these parasites.
Conclusions: Our data suggest, that T. brucei differentiation is intimately linked to the bioenergetic status of the parasite single mitochondrion, with parasites using OS levels as cues for progress through the programmed development.
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57ASM – 167 | The mitochondria-targeted antioxidant AntiOxCIN4 attenuates brain and skeletal muscle oxidative/nitrosative stress in the amyotrophic lateral sclerosis SOD1G93A mouse

D. Mena[1,2,3]; P. Soares[4]; F. Cagide[4]; S. Benfeito[4]; L. Grilo[1,2,3]; D. F. Silva[1]; P. Pinheiro[1]; E. Ferreiro[1,2]; J. Teixeira[1,2]; F. Silva[1]; F. Borges[4]; P. J. Oliveira[1,2,3]; A. I. Duarte[1,2,3]
[1]*CNC - Center For Neuroscience And Cell Biology, University of Coimbra, Coimbra, Portugal;* [2]*CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal;* [3]*IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal;* [4]*CIQUP-IMS/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal*

Background: Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease, characterized by progressive motor neuron loss, muscle weakness, atrophy, paralysis and death. Mitochondrial dysfunction and oxidative stress are implicated in ALS pathophysiology. Thus, improving mitochondrial function and/or antioxidant capacity constitutes promising therapeutic strategies to delay ALS progression. We hypothesized that the mitochondria-targeted antioxidant AntiOxCIN4 can decrease the oxidative/nitrosative stress in the brain and skeletal muscle of SOD1G93A ALS mice.
Materials and Methods: Early adult SOD1G93A ALS mice were subcutaneously injected with AntiOxCIN4 (0.1 mg/Kg/day), for 2 months. We obtained brain cortical and skeletal muscle homogenates and used colorimetry/fluorimetry-based methods to assess the effect of AntiOxCIN4 in oxidative/nitrosative stress markers nitrites and hydroperoxides, as well for measuring the activities of the antioxidant enzymes superoxide dismutase (total SOD, SOD-2) and glutathione peroxidase (GPx).
Results: AntiOxCIN4 slightly increased brain total SOD and SOD-2 activities in ALS mice, while decreasing total SOD activity in skeletal muscle ($p = 0.07$). AntiOxCIN4 also upregulated brain GPx activity ($p = 0.07$), reduced hydroperoxides levels in ALS skeletal muscle ($p = 0.009$) and

nitrites levels in ALS brain and skeletal muscle ($p = 0.002$, $p < 0.001$, respectively).

Conclusion: Our results indicate that peripherally administered AntiOxCIN4 may mitigate brain and skeletal muscle oxidative/nitrosative stress. Further studies are needed to assess whether this protection delays ALS progression.

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57ASM – 174 | Protective Effects of Equisetum ramosissimum Desf. against Palmitic Acid-Induced Lipid Accumulation and Mitochondrial Dysfunction in Human HepG2 cells

R. F. Santos Amorim[1,2]; C. Melim[1,2]; M. Pedro Marques[1,2]; C. Varela[1,2,3]; M. I. Dias[4,5]; L. Barros[4,5]; J. Teixeira[6]; P. J. Oliveira[6]; C. Cabral[1,2,7]

[1]University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Clinic Academic Center of Coimbra (CACC), Faculty of Medicine, Coimbra, Portugal; [2]University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal; [3]University of Coimbra, CIEPQPF, Faculty of Medicine, Coimbra, Portugal; [4]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus Santa Apolónia, Bragança, Portugal; [5]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; [6]CNC-Center for Neuroscience and Cell Biology, CIBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Bragança,; [7]Center for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Coimbra, Portugal

Background and aims: NAFLD is a worldwide public health concern, affecting approximately one-quarter of the adult population worldwide, and causing a significant morbidity burden with widespread social and economic implications. Equisetum ramosissimum, also known as branched horsetail, is a plant species that belongs to the Equisetaceae family. It has been used in traditional medicine for its diuretic, anti-inflammatory, and antioxidant properties. Driven by the lack of approved pharmacological therapies, we investigated the potential use of Equisetum ramosissimum Desf. Extracts from the Côa Valley (North of Portugal) in the context of NAFLD.

Method: Herein, we studied the effects of E. ramosissimum extracts: hydroalcoholic extract 80:20 (ER_EtOH80 and decoction (ER_D), 50 μg/mL; 24 h) on the human hepatoma-derived HepG2 cell line by measuring their effects on mitochondrial physiological parameters and the antioxidant defence system after exposure to supraphysiological concentrations of palmitic acid (PA, 100 uM; 24 h).

Results: E. ramosissimum extracts (ER_EtOH80 and ER_D) prevented PA-induced neutral lipid accumulation. PA decreased O2 consumption and mitochondrial membrane potential (ΔΨm), alterations that were prevented by ER_D pretreatment. In fact, ER_D modulated mitochondrial homeostasis with an increment of OXPHOS complex subunits and mitochondrial membrane proteins. Furthermore, ER_D upregulated β-oxidation pathways, as indicated by the increase in PPARα transcripts and CPT1α protein. ER_EtOH80 also showed antioxidant properties, as it antagonized CM-H2DCFDA oxidation induced by PA. Moreover, ER_EtOH80 stimulated the endogenous antioxidant defence system by increasing Nrf2 and HMOX-1 gene expression, thioredoxin protein levels, and catalase activity.

Conclusions: E. ramosissimum extracts from the Côa Valley modulate cellular metabolic activity by improving antioxidant defences and mitochondrial function, showing potential therapeutic benefits in preventing and treating metabolic disorders associated with lipid accumulation, such as NAFLD.

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57ASM – 177 | Mitochondrial genome deletions screening – Does next-generation sequencing solve it all?

M. J. Santos[1,2,3]; M. Simões[1,2,4]; S. Martins[1,2,5]; J. Durães[3,6,7]; S. Ferreira[6,7]; C. Santos[6,7]; L. Diogo[2,3,6,7]; M. do Carmo Macário[2,3,6,7]; M. Grazina[1,2,3]

[1]Laboratory of Mitochondrial Biomedicine and Theranostics, CNC – Center for Neuroscience and Cell Biology, University Of Coimbra, Coimbra, Portugal; [2]CIBB – Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [3]FMUC – Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [4]FCTUC – Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal; [5]IIIUC – Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; [6]Centro de Referência de Doenças Hereditárias do Metabolismo, MetabERN, CHUC – Centro Hospitalar e Universitário de Coimbra, EPE, Coimbra, Portugal; [7]CHUC – Centro Hospitalar e Universitário de Coimbra, EPE, Coimbra, Portugal

Introduction: OXPHOS diseases are heterogeneous and multisystemic, affecting mainly organs with higher-metabolic-rates [1, 2].

The classical ("biopsy first") diagnostic approach was based in deep clinical phenotyping and OXPHOS activity in tissues, targeting gene analysis. Recently, Next Generation Sequencing (NGS) became more accessible, leading to a "genetics first" approach [3]. Still, tissue specificity of OXPHOS should be considered when selecting biological samples for diagnosis.

We present the screening results for large mitochondrial DNA (mtDNA) deletions in 305 patients, in blood and muscle and/or liver biopsy.

Methods: Total DNA was extracted from blood, muscle, and liver biopsies by standard methods. To screen deletions, a flanking PCR was performed in six regions (3150–14,704, 3150–16,192, 3150–16,406, 7241–14,704, 8222–13,727, 8222–16,192), followed by electrophoresis. Confirmation/location were done by sequencing.

Results: From the 305 patients studied, mtDNA deletion(s) were identified in 132 patients (43%). Of these, 115 (87%) had mtDNA deletions detected only in solid tissue; in 16 (12%) there was detection in blood and muscle/liver biopsy; and in 1 patient it was detected only in blood.

Conclusions: mtDNA deletions are more likely to be found in tissue biopsies than in blood. The choice of specimen for testing is critical and increases the probability of a mtDNA molecular diagnosis [2].

The NGS approach can be a breakthrough in OXPHOS diseases' diagnosis. Our approach is based on enrichment of entire mtDNA by a single-amplicon long-range PCR prior to NGS, to detect variants and deletions simultaneously [1], allowing high specificity.

Evaluation of family history, clinical and biochemical findings, imaging/histopathological data is indispensable. Furthermore, instead of performing NGS in blood for all cases, the availability of different tissues for functional and genetic analysis is still mandatory to achieve a more precise and correct diagnosis [2].

1. https://doi.org/10.1373/clinchem.2011.181438
2. https://doi.org/10.1002/humu.22307
3. https://doi.org/10.1055/s-0037-1,603,776

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57ASM – 179 | AddFun: Adding functional genomics to genetics in mitochondrial oxidative phosphorylation diseases

M. Simões[1,2,3]; M. J. Santos[1,2,4]; S. Martins[1,2,5]; C. Alcobia Gomes[1,2,3,6]; J. Durães[4,7,8]; P. Oliveira[9]; J. C. Ferreira[10]; M. do Carmo Macário[1,4,7,8]; L. Diogo[1,4,7,8]; M. Grazina[1,2,4]

[1]CIBB – Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [2]Laboratory of Mitochondrial Biomedicine and Theranostics, CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; [3]FCTUC – Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal; [4]FMUC – Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [5]IIIUC – Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; [6]Polytechnic Institute of Coimbra, ESTeSC-Coimbra Health School-DCB, Coimbra, Portugal; [7]Centro de Referência de Doenças Hereditárias do Metabolismo, MetabERN, CHUC – Centro Hospitalar e Universitário de Coimbra, EPE, Coimbra, Portugal; [8]CHUC – Centro Hospitalar e Universitário de Coimbra, EPE, Coimbra, Portugal; [9]Service of Medical Genetics, Centro Hospitalar e Universitário de São João, Porto, Portugal; [10]Service of Paediatric Neurology, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal

Introduction: Next-Generation Sequencing (NGS) boosted discovery of new genetic defects for many diseases, including OXPHOS diseases. So far variants in >350 genes were associated with these disorders [1], which are

challenging from clinical and therapeutic perspectives. Many patients present novel variants of unknown significance. Subsequent biomolecular functional studies may clarify their pathogenic consequences, change classification and establish genetic diagnosis [2, 3].

Methods: We present data of patients (P1-P5) with novel genetic variants and corresponding functional genomics' studies: OXPHOS respiratory/glycolytic rates (Seahorse XF), enzymatic activity and assembly (BN-page), protein levels (SDS-WB), single muscle fibre assay.

P1-Leigh syndrome (40 years, male); Complex IV activity deficiency (full assembly absent), homozygous deletion (c.-11_13del, SURF1), not detected by NGS [2].

P2-Epileptic encephalopathy (8 years, male); homozygous c.882-1G>A, FASTKD2; OXPHOS decrease; reduced FASTKD2 expression and abnormal respiratory/glycolytic rates.

P3-Cardiomyopathy/nephropathy (39 years, male); c.29G>C, FASTKD2; OXPHOS decrease; reduced FASTKD2 levels.

P4-CPEO (62 years, female); multiple OXPHOS deficiency; mitochondrial DNA alterations (m.7486G>A, MT-TS1; 4977 bp del); higher levels of mutant mtDNA alterations in COX-deficient fibres [3].

P5-Polyneuropathy (15 years, female); heterozygous c.1437C>A, POLG; combined deficiency or normal OXPHOS activity/respiratory capacity (tissue variable), increased Complex I assembly; normal POLG levels.

Results: Functional studies allowed the solving of all cases. Proteins' expression levels were reduced (P1-4), confirming pathogenicity. However, pathogenicity related to POLG variant is not supported for P5. Additionally, experience showed that Sanger sequencing may overcome limitations of NGS (P1).

Conclusions: An "OXPHOS activity"-first approach can guide genetic screening and/or interpretation of variants of unknown significance. Moreover, when functional studies' results are similar in patient/controls, the pathogenicity of the variant should be inquired and a broader approach for research should be taken.

The cases presented show that functional data may be decisive for appropriate genetic counselling.

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2-https://doi.org/10.1016/j.mito.2016.10.004
3-https://doi.org/10.1016/j.nmd.2017.11.006

57ASM – 182 | Lost Gains: The long-term cardiovascular consequences of short-term exercise cessation after gestational diabetes for the maternal heart

C. Tocantins[1,2]; J. D. Martins[1]; L. F. Grilo[1,2]; M. S. Diniz[1,2]; Ó. M. Rodrigues[1]; L. Carvalho[3]; J. Stevanovic-Silva[4]; J. Beleza[4]; P. Coxito[4]; D. Rizo-Roca[5]; E. Santos-Alves[4]; M. Rios[4]; A. J. Moreno[6]; A. Ascensão[4]; J. Magalhães[4]; P. J. Oliveira[1]; S. P. Pereira[1,4]

[1]CNC – Center for Neuroscience and Cell Biology, CIBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra; 3004–531 Coimbra, Portugal; [2]PhD Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal; [3]Institute of Anatomical and Molecular Pathology, Faculty of Medicine, University of Coimbra; Coimbra, Portugal; [4]Laboratory of Metabolism and Exercise (LaMetEx), Research Centre in Physical Activity, Health and Leisure (CIAFEL), Laboratory for Integrative and Translational Research in Population Health (ITR), Faculty of Sports, University of Porto, Porto, Portugal; [5]Department of Cell Biology, Physiology & Immunology, Faculty of Biology, University of Barcelona, Barcelona, Spain; [6]Department of Life Sciences, School of Sciences and Technology, University of Coimbra, Coimbra, Portugal

Background: Gestational diabetes mellitus (GDM) is the most common pregnancy metabolic disorder. After GDM, women have a higher risk of developing non-communicable diseases, including cardiovascular disease (CVD). Mitochondrial dysfunction is often observed in CVD. Exercise is a recommended non-pharmacological strategy to improve GDM's metabolic imbalance. However, the maternal long-term cardiac consequences of GDM exercise regimens cessation after pregnancy are yet to unfold. We aimed to identify the cardiac alterations induced by exercise exclusively during GDM-pregnancy that prevailed eight weeks after exercise cessation.

Methods: Sprague–Dawley females were fed a control (C) or high-fat-high-sugar (HFHS) diet starting 7 weeks pre-gestation to induce GDM and subjected either to a sedentary (S) or exercise (Ex) behaviour during pregnancy ($n \geq 6$ /group). Oral glucose tolerance tests and gestational weight gain (GWG) were evaluated. Eight weeks after delivery and exercise cessation, plasma parameters and isolated cardiac mitochondria were analysed, and cardiac tissue evaluated through histology, immunoblotting, and enzymatic activities. T-tests were used with $p < 0.05$ considered statistically significant.

Results: The GDM impaired glucose homeostasis during gestation, but exercise prevented the HFHS-diet-induced

GWG by 30%. GDM increased plasma insulin-like growth factor-1 levels ($p < 0.05$ GDM-S; $p < 0.0001$ GDM-Ex), while exercise decreased adiponectin levels. GDM-Ex hearts had decreased inflammatory cytokines levels (TNFα, IL-6) in the cardiac tissue, despite slightly increased perivascular collagen deposition. However, GDM-cardiac mitochondria exhibited longer ADP phosphorylation depolarization lag phase ($p < 0.05$ GDM-S; $p < 0.01$ GDM-Ex) and decreased respiratory control ratio using complex-I substrates. GDM-Ex showed declined glucose-6-phosphate dehydrogenase activity in the cardiac tissue and reduced mitochondrial ATP levels.

Conclusions: Exercise performed exclusively during GDM and subsequent eight-week exercise cessation can result in potentially beneficial adaptations in the maternal organism. However, the morphological and molecular analyses, especially regarding energy metabolism, highlight the need for personalized and adapted exercise recommendations during GDM to prevent long-term cardiac consequences.

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57ASM – 183 | Maternal physical exercise during obesogenic pregnancy modulates cardiacnitric oxide signaling in young-adult offspring

S. P. Pereira[1,2*]; M. S. Diniz[1,3*]; J. D. Martins[1*]; Ó. M. Rodrigues[1]; C. Tocantins[1,3]; L. F. Grilo[1,3]; J. Stevanovic-Silva[2]; J. Beleza[2]; P. Coxito[2]; D. Rizo-Roca[4]; E. Santos-Alves[2]; M. Rios[2]; A. J. Moreno[1,5]; A. Ascensão[2]; J. Magalhães[2]; P. J. Oliveira[1]

*Equal Contribution

[1]CNC – Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal; [2]Laboratory of Metabolism and Exercise (LaMetEx), Research Centre in Physical Activity, Health and Leisure (CIAFEL), Laboratory for Integrative and Translational Research in Population Health (ITR), Faculty of Sports, University of Porto, Porto, Portugal; [3]Ph.D. Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal; [4]Cellular Biology Department, Physiology and Immunology, Faculty of Biology, University of Barcelona, Barcelona, Spain; [5]Life Sciences Department, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal

Background: Maternal obesity(MO) is a major health concern, affecting 50% of pregnancies, impairing the intra-uterine environment, and increasing the offspring's risk of cardiovascular disease(CVD). Nitric oxide(NO) is critical for vascular homeostasis. Vascular dysfunction has been observed in MO-fetoplacental units and CVD patients. However, it remains unknown whether MO affects NO signaling in the offspring's heart and if maternal exercise during MO gestation(MOEx) modulates offspring cardiac NO signaling.

Aim: Investigate the effects of MO and MOEx on young-adult offspring cardiac NO signaling.

Methods: Female pregnant Sprague–Dawley were fed a high-fat-high-sugar (HFHS; $n = 12$) or a control (C; $n = 6$) diet, 7 weeks previous to gestation. Six HFHS-fed mothers were kept sedentary (MO; $n = 6$), while six were exercised (MOEx; $n = 6$) during pregnancy. Offspring were kept on a standard chow diet without exercise. Male and female offspring (F1-C; F1-MO; F1-MOEx) were euthanized at 32-weeks-old ($n = 6$/sex), and cardiac tissue and blood plasma collected. Either unpaired t-test or Mann-Whitney test were used for statistical analysis ($p \leq 0.05$).

Results: Females F1-MO showed increased triglycerides/glucose index, an insulin resistance biomarker, compared to F1-C ($p < 0.01$), whereas it was decreased for females F1-MOEx vs F1-MO ($p = 0.01$) and males F1-MOEx vs F1-C ($p = 0.03$)). The atherogenic index (AI), reflecting CVD risk, was increased in females F1-MO vs F1-C

($p < 0.01$) and decreased in F1-MOEx vs F1-MO ($p < 0.01$). AI was decreased for males F1-MOEx vs F1-C ($p = 0.01$). Activated Akt (p-AktThr308/Akt1) was increased in females F1-MO vs F1-C ($p = 0.02$) and decreased for males F1-MOEx vs F1-C ($p = 0.03$). CAT-1, NO transporter, increased by 23% in males F1-MO vs F1-C. Nitrotyrosine, a nitrosative stress marker, was decreased in males F1-MOEx vs F1-C ($p < 0.01$).

Conclusions: Maternal obesity altered the offspring's metabolic parameters and NO precursor transport. Exercise during MO pregnancy induced sex-specific alterations in the offspring's cardiac NO signaling that prevail until the young-adult stage. Gestational exercise during MO may reduce CVD risk in MO offspring.

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57ASM – 184 | Potential protective effects of 2,4-dinitrophenol in an okadaic-acid-induced in vitro model of Alzheimer's disease

S. Cardoso[1,2,3]; P. I. Moreira[1,3,4]
[1]CNC – Center For Neuroscience And Cell Biology, University Of Coimbra, Coimbra, Portugal; [2]IIIUC – Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; [3]CIBB – Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Portugal; [4]Institute of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Alzheimer's disease (AD) is one of the most common disabling diseases among older adults. Abnormally hyperphosphorylated tau and mitochondrial dysfunction stand as major contributing factors to the onset and pathogenesis of AD. AD treatment remains an unsolved issue and an attractive approach bends to drug repositioning. In this perspective, recent evidence suggests that low doses of 2,4-dinitrophenol (DNP), a mitochondrial uncoupler formerly used in the clinic as a treatment for obesity, has neuroprotective and cognitive enhancing properties in different models of disease. Based on this, we aimed to evaluate if DNP, by directly targeting mitochondria, could play a role in mitigating AD pathophysiology.

Retinoic-acid induced differentiated SH-SY5Y cells were exposed to okadaic acid (a serine/threonine protein phosphatase type 2A inhibitor) and/or with DNP. Cell viability analysis was determined using MTT reduction assay; spectrophotometry was used to evaluate citrate synthase and aconitase activities; reactive oxygen species (ROS) production levels were assessed by fluorometry, and lipid peroxidation levels by thiobarbituric acid assay; mitochondrial bioenergetics was evaluated in a Seahorse apparatus; and western blot analysis was used to assess the protein content of pTau, and the activation status of key cellular and mitochondria homeostasis-related proteins.

Our data show that DNP was able to rescue OA-induced loss of cells viability and normalized ROS and lipid peroxidation levels, aconitase and citrate synthase activities, and oxygen consumption rate. Further, DNP reestablished mitochondrial dynamics, reduced pTau levels and restored OA-induced increased activities of extracellular signal-regulated kinase 1/2 and glycogen synthase kinase-3β.

Overall, data indicate that DNP modulates mitochondria under AD-related insults and targets several signalling pathways found to be deregulated in AD.

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57ASM-187 | NAD+ Supplementation Combined with Piezo1 Activation Restores Mitochondrial Function in Osteoblast Precursors from Aged Mice

A. Carvalho[1,2]; V. Sardão[3]; M. Almeida[4]
[1]Center For Neurosciences And Cell Biology, Cantanhede, Portugal; [2]PhD Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal; [3]Multidisciplinary Institute of Aging (MIA-Portugal), University of Coimbra, Coimbra, Portugal; [4]Division of Endocrinology and Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, University of Arkansas for Medical Sciences, Little Rock, USA

A decrease in the number of osteoblast, the bone forming cells, contributes to age-associated osteoporosis. Lower levels of NAD contribute to the decrease in bone formation with aging, as does the ability of bone to respond to exercise. Piezo1 is an ion channel critical for the response of osteoblast to mechanical loading and its effects on bone mass. Stimulation of Piezo1 with Yoda1 mimics the effects of mechanical loading and promotes mitochondria activity in osteoblastic cells. In muscle of aged humans, NAD+ abundance positively correlates with the amount of exercise and mitochondrial and muscle functioning. However, the interactions between mechanical loading,

NAD+, and mitochondria in osteoblastic cells remains poorly understood.

Bone marrow osteoblastic cells from young and old mice were cultured in osteogenic media using normal culture plates or a fluid flow chamber. Metabolic profile was measured using the Seahorse XFe96 Extracellular Flux Analyser, ATP using CellTiter-Glo Luminescent Cell Viability Assay and NAD using EnzyFluo™ NAD/NADH Assay kits. Mitochondrial membrane potential and ROS were measured with TM and MitoSOX probes.

Cells from old mice exhibited decreased mitochondrial respiration, mitochondria-derived and total ATP production, lower membrane potential and higher ROS levels. Addition of the NAD+ precursor nicotinamide riboside (NR) or Yoda1 partially recovered mitochondrial respiration while the combination of NR and Yoda1 completely restored mitochondrial function in cells from old mice to levels similar to the ones seen in cells from young mice. Fluid flow, used to mimic mechanical stimulation, increased NAD+ levels, in both young and old cells.

These findings suggest that a decrease in NAD+ levels with aging contributes to the decline in mitochondrial activity and the response to Piezo1 stimulation in osteoblast precursors. Thus, the combination of NAD+ supplementation and exercise might be advantageous in promoting bone formation and attenuating age-related bone loss in older adults.

57ASM – 188 | The mitochondria-targeted antioxidant AntiOxCIN4 protects against cardiac oxidative/nitrosative stress in the amyotrophic lateral sclerosis SOD1G93A mouse

D. Mena[1,2,3]; F. Cagide[4]; S. Benfeito[4]; P. Soares[4]; L. Grilo[1,2,3]; P. Pinheiro[5]; J. Teixeira[1,2]; F. Borges[4]; P. J. Oliveira[1,2,3]; **A. I. Duarte**[1,2,3]

[1]*MitoXT - Mitochondrial Toxicology & Experimental Therapeutics Laboratory, CNC - Center for Neuroscience and Cell Biology, University of Coimbra, UC Biotech Building, Lot 8A, Biocant Park, 3060-197 Coimbra, Portugal*

Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset neuromotor disease, characterized by progressive motor neuron loss, muscle atrophy and paralysis. Severe changes in cardiovascular function are frequently described in ALS patients, which may progress to heart failure and death. Although the precise mechanisms remain poorly understood, mitochondrial dysfunction and oxidative stress may play a pivotal role in ALS progression. Thus, rescuing mitochondrial function and/or antioxidant capacity constitutes promising therapeutic approaches against ALS progression. We hypothesized that the mitochondria-targeted antioxidant AntiOxCIN4 can attenuate cardiac oxidative/nitrosative stress in SOD1G93A ALS mice.

Methodology: Early adult SOD1G93A ALS mice were injected subcutaneously with AntiOxCIN4 (0.1 mg/Kg/day), for 2 months. We prepared heart homogenates and used colorimetry/fluorimetry-based methods to evaluate the effect of AntiOxCIN4 in oxidative/nitrosative stress markers nitrites and free thiols, as well for measuring the activities of the antioxidant enzymes superoxide dismutase (total SOD, SOD-2), glutathione peroxidase and reductase (GPx, GRed).

Results: We observed that the massively increased (by 2–3-fold) activities of total SOD, SOD-2, GRed and GPx in the hearts of AntiOxCIN4-treated ALS mice were accompanied by a slight decrease (by 1.4- and 1.3-fold) in their cardiac nitrites and free thiols' levels.

Conclusion: Our preliminary results indicate that peripherally administered AntiOxCIN4 may upregulate cardiac antioxidant defenses to counteract ALS-related oxidative/nitrosative stress. Further studies are needed to clarify if this protection delays cardiac dysfunction upon ALS progression.

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57ASM – 192 | IFNγ-induced changes in mitochondrial metabolism as a novel strategy for pancreatic cancer therapy

K. Boháčová[1,2]; Š. Boukalová[1]; B. Endaya[1]; M. Dubišová[1,2]; J. Štursa[1]; L. Werner[1]; R. Gurlich[3]; J. Neužil[1,4]; S. Štemberková Hubáčková[1,5]

[1]Institute of Biotechnology, Czech Academy of Science, Praha – západ, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Department of Surgery, University Hospital Kralovske Vinohrady and Third Faculty of Medicine, Charles University, Prague, Czech Republic; [4]School of Pharmacy and Medical Science, Griffith University, Southport, Australia; [5]Centre for Experimental Medicine, Institute of Clinical and Experimental Medicine, Prague, Czech Republic

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease for which there is currently no effective therapy. The PDAC tumours show repression of the anticancer immunity in their microenvironment. Immune checkpoint inhibitors aim to stimulate anticancer immune defence via activation of T-lymphocytes producing IFNγ, which was shown to induce cell death in malignant cells. Despite the well-established mechanism of IFNγ induced cell death, its effect on mitochondria and cellular metabolism remains elusive. Our goal was to study the role of IFNγ in regulation of mitochondrial biogenesis and its contribution to cell death.

Methods: Metabolic parameters were evaluated using flow-cytometry, confocal microscopy, and Oxygraph2k/Oroboros instrument. For in vivo studies, orthotopic and subcutaneously model of PDAC was engaged. Human and murine cell lines were grafted into NSG or C57BL/6 mice respectively. Treatment was applied by intraperitoneal injection.

Results: We show that IFNγ and IFNγ-producing immunotherapy represented by anti-PD-1 immune checkpoint inhibitor blocks aerobic glycolysis, prevents upregulation of glucose transporter and lactate secretion. Further, IFNγ regulates mitochondrial function in PDAC cells via increased autophagy of damaged mitochondria. These changes in metabolism make the pancreatic tumours more susceptible to OXPHOS inhibition by MitoTam, a potentially new anticancer agent that selectively targets highly polarized mitochondria of malignant cell, where induces electrochemical imbalance resulting in cell death. This combined therapy showed stabilization or rejection of tumours and significantly prolonged survival in all tested animals compared to immunotherapy or MitoTam alone.

Conclusion: IFNγ produced during immunotherapy regulates mitochondrial biogenesis in PDAC with metabolic shift to oxidative phosphorylation and improves tumour microenvironment. Our study therefore suggests a novel promising strategy for treatment of pancreatic cancer by simultaneous targeting of mitochondrial metabolism and immune checkpoints.

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57ASM – 200 | Mitochondrial morphology and metabolism in pancreatic β-cell form IF1 KO mice

B. Bazila[1,2]; P. Jezek[1]; A. Dlasková[1]

[1]Laboratory of Mitochondrial Physiology, Czech Academy of Science, Institute of Physiology, Prague, Czech Republic; [2]Department of Cell Biology and Pathobiology, Charles University, Prague, Czechia

Introduction: Inhibitory factor 1 (IF1) is an endogenous regulator of ATP synthase. Previous studies have established that IF1 binds and inhibits ATP synthase upon a large decrease in membrane potential (ΔΨm) when ATP synthase reverses and hydrolyses ATP, preventing total ATP depletion. What is yet to be established is how IF1 regulates ATP synthesis under normal physiological conditions when the enzyme works in the regular forward mode. We have previously shown that IF1 regulates cellular respiration, ATP levels, and glucose-stimulated insulin secretion in model pancreatic β-cells (INS-1E) under normal physiological conditions 1, 2.

Aim: The aim of this study was to investigate cellular metabolism and mitochondrial morphology in pancreatic beta cells in-situ in pancreatic islets isolated from IF1 KO mice.

Material and Methods: We studied ATP levels in pancreatic islets treated with low and high glucose concentrations with inactive/active glucose-stimulated insulin secretion (GSIS). Insulin and glucagon expression was also studied by immunohistochemistry and compared between pancreatic islets of IF1 KO and WT mice. Moreover, using super-resolution microscopy, mitochondrial morphology was successfully visualized in pancreatic islets under different metabolic conditions.

Conclusion: Altogether, the obtained data provided further insight into the emerging role of IF1 in the metabolism of pancreatic beta cells. The project was funded by Grant Agency of the Czech Republic (No. GA22-02203S to Ing. Andrea Dlasková, Ph.D.).

References

1. Kahancová, A., Sklenář, F., Ježek, P. et al. Overexpression of native IF1 downregulates glucose-stimulated insulin secretion by pancreatic INS-1E cells. Sci Rep 10, 1551 (2020).
2. Kahancová A, Sklenář F, Ježek P, and Dlasková A. Regulation of glucose-stimulated insulin secretion by ATPase inhibitory factor 1 (IF1). FEBS Lett 592: 999–1009, 2018.

57ASM – 204 | Impact of maternal obesity and reduced nutrition on fetal baboon heart development: Transcriptional modulation of cardiomyocyte maturation, proliferation and enlargement

L. F. Grilo[1,2,3,4]; S. P. Pereira[1,5]; P. J. Oliveira[1]; C. Li[6]; K. Lange[7]; J. P. Glenn[8]; K. D. Zimmerman[3,4]; P. W. Nathanielsz[6]; L. A. Cox[3,4,8,9]

[1]Center For Neuroscience And Cell Biology, University of Coimbra, Coimbra, Portugal; [2]Ph.D. Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal; [3]Center for Precision Medicine, Wake Forest University School of Medicine, Winston-Salem, USA; [4]Section on Molecular Medicine, Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, USA; [5]Laboratory of Metabolism and Exercise (LaMetEx), Research Center in Physical Activity, Health and Leisure (CIAFEL)-Faculty of Sports – University of Porto (FADEUP) and Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal; [6]Texas Pregnancy & Life-Course Health Research Center, Department of Animal Science, University of Wyoming, Laramie, USA; [7]Southwest Research Institute, San Antonio, USA; [8]Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, USA; Section on Comparative Medicine, Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, USA

Introduction: Healthy fetal development depends on adequate maternal nutrition. Maternal obesity (MO) and nutrient reduction (MNR) are common during pregnancy and have been associated with offspring's increased cardiovascular disease (CVD) risk. The modulation of maternal nutrition disrupts the timely flow of hormones, nutrients, and metabolites during heart development. Offspring born to MO and MNR show cardiac dysfunction at early-life stages. However, the mechanisms occurring in the fetal heart underlying this association remain unclear. We investigated the effects of modulating maternal nutrition on fetal heart development.

Materials and Methods: Female baboons (Papio sp.) were fed with high-fat/high-fructose diet starting at least four-months before pregnancy (MO; $n = 5$), 30% regular-diet reduction starting one-month after conception (MNR; $n = 6$), or regular-diet throughout the study (C; $n = 6$/cohort). At 90% of the gestation (165 days, term ~185 days), c-section was performed and fetal heart left ventricle collected. Untargeted microarray assays were done followed by pathway enrichment analysis. Cohorts were compared through meta-analysis.

Results: Decreased gene expression of cardiomyocyte maturation markers were found in MO and MNR. MNR upregulates transcription pathways promoting cellular growth, likely mediated by growth factors, and cardiomyocyte enlargement. MO inhibits cellular proliferation suppression contributing to increased cardiomyocyte number but less specialized. Unbalanced cell proliferation and growth due to maternal nutrition are associated with fewer mature cardiomyocytes in a transcriptionally-regulated way in the fetal heart. Inflammation and G-protein-coupled receptor signalling are upregulated with MNR and downregulated in MO, highlighting that mechanism dysregulation may occur in opposite directions for MNR and MO.

Conclusions: MO and MNR affect fetal primate heart by modulating cardiomyocyte proliferation and growth through transcriptional changes, leading to compromised cardiac function and response to stress in offspring. These maladaptations in the heart may contribute to increased CVD predisposition. This provides insight into the role of MO and MNR in fetal maladaptations and CVD.

57ASM – 207 | Targeting aspartate synthesis dependency in cancer

M. Milosevic[1,2]; K. Dmytruk[1,3]; S. Magalhães Novais[1]; A. Alghadi[1,2]; K. Rohlenova[1]; J. Rohlena[1]

[1]Institute of Biotechnology, Czech Academy of Science, Prague, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Department of Molecular Biology and Biotechnology, Kyiv Academic University, Kyiv, Ukraine

Introduction: To form tumours, cancer cells need a biosynthetic input from the mitochondrial oxidative phosphorylation (OXPHOS) system to generate pyrimidines. In the complete absence of OXPHOS cancer cells cannot form tumours in mice. Beside supporting de novo pyrimidine synthesis directly via dihydroorotate dehydrogenase, OXPHOS also provides NADH/NAD+ recycling at complex I, which allows production of aspartate, a pyrimidine precursor, through glutamate oxaloacetate transaminase

1/2 (GOT1, GOT2). However, it is unclear if complex I deficiency and aspartate synthesis deficiency have similar impact on tumour growth.

Methods: B16 murine melanoma cell line was used to generate knock-out models (Got1/2, Ndufv1) using CRISPR-Cpf1 system. Tumours were generated by subcutaneous injection of B16 cell line in C57BL/6 mice and collected after reaching ethical culling point of 1000 mm3. We analysed metabolism of these tumours by metabolic MALDI imaging, while metabolic analysis of intracellular levels of metabolites in cells cultured in vitro were detected using GCxGC-TOF.

Results and conclusion: To address the question if complex I deficiency and aspartate synthesis deficiency have the same impact, we ablated aspartate synthesis enzymes (GOT1 KO, GOT2 KO, GOT1-2 dKO), and complex I (NDUFV1 KO) in murine cancer cells and assessed how they form tumours in syngeneic mice. Unlike complex I knockout, which severely hampered tumour formation, deficiency of aspartate synthesis enzymes had little or no effect. Hence, complex I deficiency is not equivalent to deficiency of aspartate synthesis, despite aspartate being similarly reduced in both conditions. In addition, GOT1-2 dKO sensitized cells to inhibition of proliferation with aminooxyacetic acid, a pan-transaminase inhibitor, in vitro and in vivo, suggesting metabolic dependencies on transamination pathway in GOT1-2 dKO. We are currently investigating how GOT1-2 dKO cancer cells obtain aspartate and form tumours.

57ASM – 208 | Mitochondria Pyrimidine de novo synthesis in endothelial cells: An overlooked target?

P. Hyrossova; I. Milislav; S. Novais; M. Milosevic; J. Rohlena; K. Rohlenova

Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic

Introduction: Blood vessels are a crucial component of tumor environment. Tumors stimulate vessel growth, angiogenesis, to gain oxygen and nutrients. Metabolism of endothelial cells (ECs) is rewired in tumors, and tumor ECs upregulate pyrimidine de novo synthesis (PDNS). However, whether and how endothelial PDNS supports tumorigenesis is unknown. We hypothesized that endothelial PDNS affects tumor environment directly by providing pyrimidines to cancer cells or indirectly by stimulating angiogenesis, making systemic resources more accessible to cancer cells. This would negatively impact nucleotide-targeted therapy in cancer.

Aims: We aim (i) to elucidate how ECs metabolically communicate with cancer cells in tumors; (ii) to assess if a blockade of endothelial PDNS compromises angiogenesis.

Methods: To study EC adaptations to PDNS defect during tumorigenesis, we developed an experimental model that combines inducible mice with endothelial-specific PDNS deficiency (by a DHODH KO) with orthotopic lung tumors. In this model, we apply a multi-omics approach using single cell and spatially resolved transcriptomics and metabolomics. To assess how PDNS blockade affects angiogenic properties of ECs, we tested the migratory and sprouting capacity of PDNS-deficient primary ECs in vitro.

Results: PDNS deficiency, induced genetically or pharmacologically, resulted in decreased proliferation, migration and sprouting of primary ECs in vitro. Interestingly, while the proliferation defect can be rescued by uridine, the migration and sprouting defects not, suggesting an unknown, nonmetabolic function for DHODH. We are currently analyzing the single cell and spatial transcriptomics to assess the adaptation of ECs to PDNS blockade in vivo.

Conclusions and Funding: PDNS deficiency compromises angiogenesis in vitro. The in vivo mouse model of endothelial-specific DHODH KO will allow us to search for targetable vulnerabilities of ECs improving the efficacy of PDNS-targeted treatments.

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57ASM – 230 | Base excision repair in mitochondria, mitochondrial DNA copy number and telomere length and link to colorectal cancer outcome

S. Vodenkova[1,2]; K. Tomasova[1,2]; P. Hanak[1]; N. Danesova[1]; L. Vodickova[1,2,3]; R. Godschalk[4]; S. A. S. Langie[4]; **P. Vodicka**[1,2,3]

[1]*Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic;* [2]*Biomedical Center of the Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic;* [3]*Institute of Biology and Medical Genetics of the First Faculty of Medicine, Charles University, Prague, Czech Republic;* [4]*Department of Pharmacology & Toxicology, School for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, The Netherlands*

The dysfunction of mitochondria is one of the cancer hallmarks. Mitochondria evince a limited DNA repair capacity and compensate for damage by increasing the mitochondrial DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN in cancer have, however, reported

ambiguous results. In this context, base excision repair (BER) is the major repair pathway for oxidative DNA damage removal, taking place in nuclei and mitochondria. Both in nuclear and mitochondrial DNA (mtDNA), the repair process is initiated by DNA glycosylases, such as 8-oxoguanine DNA glycosylase 1 (OGG1), that recognize and incise N-glycosidic bonds between the damaged base and deoxyribose. MtDNA is exposed to reactive oxygen species more than nuclear DNA, in particular, due to its proximity to the electron transport chain. Functional BER, keeping mtDNA intact, is necessary for the proper cell energetic metabolism and for preventing mtDNA mutations leading to carcinogenesis.

Telomere shortening has a dual role in tumorigenesis. It promotes cancer initiation by inducing chromosomal instability, while telomere length (TL) maintenance characterized by telomerase expression is required for cancer cell proliferation and tumour growth. Similar to mtDNA-CN, the reports on TL so far available are contradictory.

MtDNA-CN and TL are highly variable across cell types but maintained within a constant range according to the specific tissue type. It has been demonstrated that mitochondrial biogenesis and energy production were decreased in telomerase-deficient mice with severe telomere dysfunction. It has been hypothesized that telomere alteration affects both the oxidative defence mechanisms and mitochondrial functions. The deregulation of the telomere-mitochondria axis may trigger carcinogenesis. We investigated mitochondria and telomere changes in colorectal cancer (CRC). Our study aimed to look closely at mtDNA-CN, mtDNA damage, TL and the expression of mitochondrial transcription factor A and telomerase reverse transcriptase in association with CRC patient outcomes. We analyzed deep-frozen tumour tissue, adjacent nontumour tissue and blood from 163 untreated sporadic CRC patients. Currently, the experiments are in progress and the data will be presented at the symposium. Here we also present pilot studies on the *in vivo* determination of BER in mtDNA. We believe that these data may contribute to the current understanding of CRC, by identifying the role of BER in mtDNA, mtDNA-CN and TL in CRC pathogenesis.

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57ASM – 224 | Activation of the integrated stress response rewires cardiac metabolism in Barth syndrome

J. Dudek[1]; I. Kutschka[1]; E. Bertero[1,2,3]; C. Wasmus[1]; K. Xiao[4,5]; L. Yang[6]; X. Chen[7]; Y. Oshima[7]; M. Fischer[8]; W. Schmitz[9]; P. Rehling[10]; T. Thum[4,5,11]; T. Higuchi[7]; J. Rabinowitz[12]; C. Maack[1]

[1]Deutsches Zentrum für Herzinsuffizienz Würzburg, Universitätsklinikum Würzburg, Am Schwarzenberg 15, 97078 Würzburg, Germany; [2]Department of Internal Medicine, University of Genova, Genova, Italy; [3]Cardiovascular Disease Unit, IRCCS Ospedale Policlinico San Martino – Italian IRCCS Cardiology Network, Genova, Italy; [4]Institute of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Carl-Neuberg-Str. 1, Hannover 30625, Germany; [5]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Nikolai-Fuchs-Straße 1, Hannover 30625, Germany; [6]Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, 320 Yueyang Rd., Shanghai 200031, China; [7]Department of Nuclear Medicine, Universitätsklinikum Würzburg, Oberduerrbacher Strasse 6, 97080 Würzburg, Germany; [8]Division of Pediatric Cardiology and intensive Care, University Hospital LMU Munich, Marchioninistr. 15, 81377 München, Germany; [9]Department of Biochemistry and Molecular Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany; [10]University Göttingen, Institute of Biochemistry and Molecular Cell Biology, Humboldtallee 23, 37072 Göttingen, Germany; [11]REBIRTH Center for Translational Regenerative Medicine, Hannover Medical School, Carl-Neuberg-Str. 1, Hannover 30625, Germany; [12]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, USA

Introduction: Barth Syndrome (BTHS) cardiomyopathy is an inherited disease caused by defects in the enzyme tafazzin, which is involved in the biogenesis of the mitochondrial phospholipid cardiolipin. BTHS patients present with cardiomyopathy, skeletal myopathy, growth retardation and neutropenia. We previously identified defects in the respiratory chain, Krebs cycle and in mitochondrial Ca2+-signaling. Interestingly, despite oxidation of pyridine nucleotides under increased workload conditions, no oxidative stress developed in the heart. Here, we describe fundamental changes in metabolism and investigate how metabolic rewiring compensates for the mitochondrial defects.

Materials and Methods: Mice with an inducible knockdown (KD) of tafazzin (Taz) and BTHS patient-derived induced pluripotent stem cells were analyzed at the transcriptional, protein and metabolic flux level by integrating

unbiased genetic approaches, in vitro respirometry and in vivo metabolic flux analysis with positron emission tomography (PET) in vivo.

Results: Cardiac uptake and oxidation of fatty acids were reduced due to a downregulation of fatty acid transport and oxidation enzymes, while glucose uptake was increased. Unbiased transcriptomic analyses revealed a strong upregulation of one-carbon metabolism, which diverts glucose from glycolytic oxidation towards the biosynthesis of serine. We identified the eIF2a/ATF4 axis of the integrated stress response as the driver of these transcriptional changes that fuel the biosynthesis of glutathione. Positron emission tomography (PET) revealed a strong upregulation of the glutamate/cystine antiporter xCT in vivo, which mediates cardiac cystine import required for glutathione synthesis. Increased glutamate uptake facilitates anaplerotic replenishment of the Krebs cycle, sustaining energy production and antioxidative pathways.

Conclusion: Mitochondrial dysfunction in energy metabolism and redox homeostasis is compensated by ATF4-driven rewiring of cardiac substrate utilization. Metabolic changes included upregulation of one-carbon and amino acid metabolism to sustain energy production and antioxidation. These insights provide new opportunities for diagnostics, dietary considerations and pharmacological targeting in a life-threatening disease orphaned from effective treatments.

57ASM – 236 | Horizontal transfer of mitochondria in a mouse model of glioblastoma

O. Vaňátko[1]; P. Brisudová[2]; R. Zobalová[2]; M. Kubísková[1]; A. Foltýnová[1]; N. Ziólkowska[3]; D. Jirák[3]; J. Neužil[2]; M. Anděrová[1]

[1]Institute of Experimental Medicine, Czech Academy of Sciences, Department of Cellular Neurophysiology, Prague, Czech Republic; [2]Institute of Biotechnology, Czech Academy of Sciences, Laboratory of Molecular Therapy, Vestec, Czech Republic; [3]Institute for Clinical and Experimental Medicine, Department of Diagnostic and Interventional Radiology, Prague, Czech Republic

Glioblastoma is a malignant type of tumor characterized by fast progression, extreme recurrence rate and poor prognosis. Currently, there is no available treatment for the disease. A recently described feature in tumors is horizontal transfer of mitochondria (HTM) - a process where there is an exchange of mitochondria between tumor cell and surrounding nonmalignant cells. HTM restores cellular respiration and proliferation, as these processes are negatively affected by accumulation of damage to mitochondrial DNA in tumor cells. Details and mechanisms of HTM are currently largely unknown. Similarly, the involvement of different brain cell types in HTM remains unexplored. The aim of this work is to elucidate whether and to which extent specific brain cell types (astrocytes, microglia, NG2 glia, pericytes) participate in HTM in a mouse model of glioblastoma. In this project, we employ mouse models of the cre-lox system for visualization of mitochondria *in vivo*. Five different mouse strains expressing cre recombinase under cell type-specific promoters (Aldh1l1, Tmem119, Cspg4, Pdgfra, Pdgfrb) are crossed with reporter strain, resulting in expression of green fluorescent protein targeted into outer mitochondrial membrane. We also employ a murine GL261 glioblastoma cell line devoid of mtDNA (ρ0 cells), as horizontal transfer of mitochondria is necessary to restore mitochondria-related processes in these cells after grafting into the brain. Immunohistochemical and FACS analyses are used as the primary methods for evaluation of mitochondrial transfer. The data will be further expanded with the use of single-cell and spatially resolved transcriptomics, as well as *in vivo* imaging. As glioblastomas are currently a type of tumor with fast progression, no cure and almost 100 % lethality, identification of possible cellular and molecular targets that may be used for development of novel therapeutic agents for glioblastoma treatment is of special importance. Results from this project will contribute to the understanding on the topic of HTM and provide an insight into the biology of glioblastoma in general.

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57ASM – 235 | Fueling for a trip: Bioenergetic determinants of cancer cell migration

M. Elgendy[1,2,3]

[1]Institute for Clinical Chemistry and Laboratory Medicine, University Hospital and Faculty of Medicine, Technische Universität Dresden, Dresden, Germany; [2]Medical Clinic I, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; [3]Laboratory of Cancer Cell Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Cancer cells reprogram their metabolism to meet the high metabolic demands fueling fast growth. Glycolysis and oxidative phosphorylation (OXPHOS) are the main energy-producing pathways. Until recently, tumor metabolism was synonymous to increased glycolysis (Warburg effect). This hypothesis has been challenged: Increased glycolysis is not a universal feature of all tumors; even in glycolytic

tumors, OXPHOS is not completely shut down; finally, glycolysis inhibitors showed limited clinical success.

An emerging theme is that some cancer cells exhibit "metabolic flexibility" and engage both glycolysis and oxidative phosphorylation (OXPHOS) to adapt to metabolic challenges. However, very little is known about the molecular mechanisms that coordinate the switch among different metabolic pathways and implications on metastasis.

We metabolically profiled a wide panel of melanoma cells according to their bioenergetic dependency into glycolysis- or OXPHOS-dependent cells. In a second step, we examined the response of glycolytic and OXPHOS-dependent cells to the inhibition of their wired bioenergetic program. While some cancer cells suffered a metabolic crisis and a drop in ATP and eventually cell death, others were able to adapt and tolerate the inhibition of their wired bioenergetic program. Further analysis showed that the resistance of the latter cells correlated tightly with their ability to shuffle between OXPHOS and glycolysis to circumvent the inhibition of either process. Differential capacity of cancer cells to switch among alternative bioenergetic pathways may reflect varying potential of metabolic plasticity. Interestingly, bioenergetic dependency of melanoma cells significantly correlated with their migration capacity. Subjecting cells from both categories to migration assays showed a marked difference: In contrast to OXPHOS-dependent cells, glycolytic cells were highly migratory, invasive and exhibited higher metastatic potential in vivo. Inhibition of glycolysis impeded the migration of glycolytic cells. Interestingly, forcing a switch to glycolysis in metabolically plastic OXPHOS-dependent cells using clinically available OXPHOS inhibitors was associated with enhanced migration. Investigating how glycolysis may promote migration, we identified a crucial role for lactic acid, a byproduct of glycolysis as treating metabolically plastic -but not committed- OXPHOS-dependent cells with lactate was sufficient to enhance their migration.

Metabolic plasticity of tumors gives the rationale to attempt strategies simultaneously targeting both glycolysis and OXPHOS. As one may expect such approaches may be complicated by the possible toxicity on normal cells. We have described a therapeutic approach to target metabolic plasticity of tumors combining intermittent fasting and metformin (Elgendy et al, Cancer Cell 2019). This approach was both effective in impeding tumor growth as well as tolerated by mice. This approach shows promise in ongoing clinical trial (NCT03709147).

In conclusion, our work sheds unpresented light into the processes of bioenergetic dependency and plasticity with correlation with metastatic potential and finally describes clinically relevant therapeutic approaches to target this plasticity using translatable metabolic strategies (Elgendy et al., Cancer Cell 2019) that offer promise in ongoing clinical trials (NCT03340935; NCT03454282; NCT03709147) and may as well be tailored to impede metastasis.

57ASM – 225 | Assessing mitochondrial substrate utilization *in vitro* and *in vivo* with ^{13}C-enriched substrates and ^{13}C NMR spectroscopy

J. G. Jones
University of Coimbra, Portugal

The oxidation of acetyl-CoA by the Krebs cycle is a major pathway for the generation of reducing equivalents and ATP in aerobic tissues. The sources of mitochondrial acetyl-CoA for oxidation can be influenced by both substrate and oxygen availability and also by strong metabolic control points such as the pyruvate dehydrogenase complex and transport of long-chain fatty acids into the mitochondria via the carnitine/acyl carnitine shuttle.

Meanwhile, the yield of reducing equivalents and/or ATP per mol of acetyl-CoA oxidized is dependent on the integrity of the electron transport chain and inner mitochondrial membrane, as well as the function of the F_1F_O ATP synthase. Since the pathophysiology of many diseases involves alterations in substrate and/or oxygen availability and/or mitochondrial dysfunction, the analysis of substrate selection and can provide new insights into tissue and organ function in these settings.

^{13}C NMR isotopomer analysis is a powerful and versatile tool for studying mitochondrial substrate selection for oxidation. Oxidation of up to three different ^{13}C-enriched substrates can be simultaneously monitored under both steady-state and nonsteady-state metabolic conditions. In addition, the methodology can resolve ^{13}C-entry into the Krebs cycle via acetyl-CoA and via anaplerotic pathways. This presentation will explain the basis of this methodology with examples taken from both isolated perfused tissues and from *in situ* human liver.

57ASM – 242 | Unlocking the potential of the mammalian electron transport chain

J.B. Spinelli
University of Massachusetts Chan Medical School, Program in Molecular Medicine, Worcester MA 01605

Abstract: The mammalian electron transport chain (ETC) comprised a series of redox reactions that serve many bioenergetic and biosynthetic functions. Central to the mammalian ETC is the electron carrier ubiquinone (CoQ-10), which directs the flow of electrons in the ETC

through its reduction potential. The reduction potential of ubiquinone is higher than that of electron inputs such as NADH, allowing ubiquinone to accept electrons from these molecules in a thermodynamically favorable manner. Moreover, its reduction potential is lower than cytochrome C and oxygen, allowing ubiquinol to transfer its electrons to these molecules in a thermodynamically favorable manner. We recently discovered that mammalian mitochondria can also utilize fumarate as a terminal electron acceptor, whereby electrons from ubiquinol are transferred onto fumarate, generating succinate. In spite of the fact that fumarate has a lower reduction potential than ubiquinone, we found that ubiquinol accumulation is sufficient to drive the succinate dehydrogenase (SDH) complex in reverse, enabling ubiquinol-mediated fumarate reduction. Paradoxically, in contrast to cultured cells, certain mammalian tissues can use fumarate as a terminal electron acceptor independently of ubiquinol accumulation. Thus, we hypothesized that a novel mechanism drives the use of fumarate as a terminal electron acceptor *in vivo*. In this seminar, we elucidate the mechanism through which mammalian tissues divert electrons onto fumarate as a terminal electron acceptor.

57ASM – 243 | Metabolic remodeling in regenerating liver

B.B. Endaya; L. Kucera; L. Thi Dan Diem; G. Lopes Oliveira; J. Neuzil
Institute of Biotechnology of the Czech Academy of Sciences

Introduction: Liver is unique in its ability to regenerate upon injury and tissue loss. This process of regeneration is extremely fast, thereby inducing a rapid compensatory hyperplastic response to normalize lost functional capacity. To cater to this need for fast regeneration, the liver undergoes a highly coordinated series of metabolic 'switches' at different stages of regeneration to effectively 'shuttle and shuffle' metabolites into anabolic pathways. To date, many of these 'switches' remain undefined. Our aim is to identify the dynamic metabolic changes and how they are regulated to support rapid liver parenchymal reconstitution. Understanding this process may bring potential benefit to pathologies of liver with decreased capacity to regenerate.

Materials and methods: We determined metabolic and phenotypic changes at different stages of liver regeneration after 40% hepatectomy, using in vivo molecular and histological imaging techniques, in combination with integrated targeted proteomics and spatial metabolomics (MALDI).

Results: Marked acute steatosis-like phenotype within 24 h of regeneration constituted an early hyperplastic response followed by exponential mitotic replication immediately post-steatosis, signifying lipids as a potential source to fuel regeneration and biomass synthesis. Interestingly, a metabolic switch in the urea cycle promoted a shift from ammonia detoxification to its diversion into metabolic pathways converting it to substrates for amino acids and de novo nucleotide syntheses.

Conclusion: To sustain the high level of proliferation in regenerating liver, metabolism switches to anabolic pathways coupled with metabolic recycling of ammonia to support rapid liver biomass synthesis.

57ASM – 244 | Mitochondrial movement between mammalian cells: the cutting edge of fundamental bioenergetic knowledge

M.V. Berridge[1]; J. Neuzil[2,3]
[1]*Malaghan Institute of Medical Research, Wellington, New Zealand;* [2]*School of Pharmacy and Medical Science, Griffith University, Southport, Qld, Australia;* [3]*Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic*

The mammalian mitochondrial genome is a small compact, circular double-stranded DNA molecule comprising 37 genes, 13 of which encode essential respiratory complex subunits. Since the archaeal endocytic event that gave rise to mitochondria about 2.4 billion years ago, mitochondria have evolved to play a central role not only in cell metabolism but also in cell signaling, survival and death. Intercellular mitochondrial transfer has been identified in some plants and fungi, and in bdelloid rotifers, but until recently, examples in mammals have been sparse and limited to the last decade. The ability of organelles to transfer between mammalian cells in culture was originally shown in 2004, and genetic evidence for periodic transfer in a canine transmissible venereal tumor demonstrated a few years later. Evidence for mitochondrial transfer following lung damage followed and transfer unequivocally shown in mouse melanoma and breast cancer models in our laboratories in 2015 where tumor cells devoid of mitochondrial (mt)DNA acquired mitochondria from non-tumor cells in the microenvironment. After a long lag period of about 20 days, tumors that were functionally identical to the original tumor formed. With each tumor type, proof of concept involved genetic polymorphisms in mtDNA where the genotype was that of the host mouse. This presentation will describe the unlikely paradigm-altering journey that led to the realization that protein-coding genes in mammalian somatic cells are not always constrained

within the cell of origin. Extensive evidence indicates the intercellular mitochondrial transfer is a common physiological phenomenon that is exacerbated under certain stress conditions, and in many diseases in animal models and in the clinic.

57ASM – 245 | Biogenesis and function of miniature mitochondria in the intestinal pathogen *Giardia intestinalis*

P. Doležal
Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, Vestec, Czech Republic

Introduction: *Giardia intestinalis* is the most common intestinal parasite of humans across the world. The parasitic lifestyle in the anoxic environment led to the extreme adaption of the metabolism of this unicellular eukaryote. Its mitochondria were reduced to organelles called as mitosomes that perform only a single metabolic role, the assembly of Fe-S clusters via ISC pathway.
Methods: We introduced CRISPR/Cas9 system to manipulate tetraploid genome of *G. intestinalis* and used quantitative proteomics to study the function and the interactome of mitosomal proteins. We also employed live-cell and FIB/SEM microscopy to study the dynamics and the inheritance of the mitosomes.
Results and conclusion: We have been able to show that mitosomes are extremely stable organelles that do not undergo fusion and their division is completely synchronized with mitosis. About forty proteins constitute the entire proteome and these proteins either participate in the biogenesis of the organelle or mediate the formation of Fe-S clusters. Interestingly, the clusters formed in mitosomes are only required outside the organelle because there are no homologs of respiratory complexes or other cluster-containing proteins. We propose that mitosomal homologue of IscA2 could be involved in the export of Fe-S cluster for the cytosolic and nuclear proteins. Mitosomes thus represent a unique experimental system for studying the non-mitochondrial role of the ISC pathway.

57ASM – 246 | *Trypanosoma brucei*, a master of metabolic disguise

E. Doleželová [1]; M. Kunzová[1,2]; B. Panicucci; A. Zíková[1,2]
[1]*Institute of Parasitology, Biologe Centre, Czech Academy of Sciences, 370 05, České Budějovice, Czech Republic;*
[2]*Faculty of Science, University of South Bohemia, 370 05, České Budějovice, Czech Republic*

Introduction: *Trypanosoma brucei*, the causative agent of African trypanosomiasis, is a master of metabolic adaptations to its environment. This unicellular parasite, which undergoes a complex digenetic life cycle in the insect and mammalian hosts, metabolically adapts to the host environment and the nutrients offered. In terms of energy metabolism, the insect forms of the parasite consume mainly amino acids (e.g. proline, threonine), which are oxidized in its single mitochondrion to succinate, acetate and alanine generating ATP by both oxidative and substrate phosphorylation. In contrast, the bloodstream form (BSF) generates most of its cellular ATP in the cytosol by glycolysis, excludes its mitochondrion as the cell´s powerhouse and uses F_oF_1-ATP synthase in the reverse mode to maintain mitochondrial membrane potential.
Methods: Using cell-based and -omics approaches we thoroughly mapped the programmed metabolic remodeling of the parasite and identified hallmarks of this process.
Results and conclusion: One of the most important features of the differentiation of the parasite insect forms was an increased level of mitochondrial reactive oxygen species (mROS), known signaling molecules. When mROS were reduced by genetically introduced scavengers (catalase, mitochondrial catalase, superoxide dismutase), parasite differentiation was severely impaired. In contrast, when mROS production was artificially increased, the parasite differentiated more efficiently to metabolically quiescent form. We linked mROS production to higher proline consumption, which generates high levels of NADH, suggesting involvement of NADH dehydrogenases in mROS production. We have also shown that mROS, rather than an increase in the AMP /ATP ratio, is critical for activation of AMP-activated protein kinase (AMPK), a cellular energy sensor that promotes cell survival under environmental stress. Our data suggest that the parasite has adapted generic stress pathways to drive its programmed development. Finally, I will highlight the importance of *T. brucei* as a model organism that provides a simplified system with a new perspective for studying complex mitochondrial contributions in the cell.

57ASM – 247 | Mitochondrial ROS formation in cardiac physiology and disease

Nina Kaludercic
University of Padua

Mitochondrial reactive oxygen species (ROS) play a major role in tissue homeostasis and contribute to the development of several cardiovascular pathologies. Within mitochondria, ROS can be produced at several sites. One of the most prominent sites for ROS formation in the mitochondria is monoamine oxidases (MAOs), flavoenzymes located in the outer mitochondrial membrane. MAOs are responsible for the degradation of neurotransmitters and biogenic amines, and during this process, they generate hydrogen peroxide, aldehydes and ammonia, species that can target mitochondria and induce mitochondrial dysfunction and cardiomyocyte death. Indeed, accumulating evidence highlighted the role of MAOs in cardiovascular diseases, such as ischemia/reperfusion, heart failure, diabetes and doxorubicin-induced cardiotoxicity. Nevertheless, MAO-dependent ROS formation appears to be also important for signaling in physiological conditions, i.e. during cardiomyocyte differentiation. Here, I will present findings linking MAO activation to cardiac alterations in physiology as well as in pathological conditions, such as cardiomyopathy associated with Duchenne muscular dystrophy.

57ASM – 248 | Signaling functions and assembly of respiratory complex II

G. Cecchini[1]; E. Maklashina[1]; P. Singh[2]; T.M. Iverson[2]; S. Balintova[3]; S. Dvorakova[3]; J. Rohlena[3]; J. Neuzil[3,4]
[1]*University of California in San Francisco, CA, USA;* [2]*Vanerbilt University, Nashville, TN, USA,* [3]*Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic;* [4]*School of Pharmacy and Medical Science, Griffith University, Southport, QLD, Australia*

Introduction: Complex II (CII) (*i.e.* succinate:quinone oxidoreductase/succinate dehydrogenase, bacterial fumarate reductase) is both a part of the mitochondrial respiratory chain and a key component of the TCA (Krebs) cycle. In mammals, the enzyme is a heterotetramer bound to the mitochondrial inner membrane. The four structural subunits of CII include a flavoprotein (SDHA), iron-sulfur protein (SDHB) and two hydrophobic membrane anchors (SDHC and SDHD). It is well understood that CII contributes to essential metabolic functions; but it has only more recently been shown that complex II activity also contributes to a range of biological processes including inflammation, epigenetics, cell fate and metabolic control. These latter functions of CII largely relate to the catalytic activity of complex II that occurs from the dicarboxylate active site of the flavoprotein (SDHA) and iron-sulfur protein (SDHB) subunits of CII and the products of this reaction succinate and fumarate. The assembly of CII into a catalytically functional enzyme is not a trivial process in mammals and requires at least four additional assembly factors (SDHAF1-SDHAF4).
Materials and methods: We followed the assembly of CII using bacterial models and also heterologous expression of the human proteins in bacteria and human cell lines. Protein assembly was analyzed by a combination of protein chemistry, x-ray crystallography, cryo-EM and pull-down assays.
Results: In order to proficiently incorporate the covalently-bound FAD cofactor into the apo-SDHA protein; FAD, the SDHAF2 assembly factor and a dicarboxylate such as fumarate are required. This results in a very tight SDHA-SDHAF2 complex harboring covalently-bound FAD. We show that another assembly factor SDHAF4 is required to release SDHAF2, so that the SDHA subunit is able to interact with SDHB. X-ray crystal structures of the SDHA-SDHAF2-SDHAF4 and SDHA-SDHAF4 complex enable us to describe a mechanism of how this process proceeds.
Conclusion: The binding of SDHAF4 to the SDHA-SDHAF2 complex displaces SDHAF2 from complex resulting in unmasking of the protein binding site where SDHB is now able to bind to SDHA.

57ASM – 249 | Turnover and quality control of mitochondrial respiratory complexes

Karolina Szczepanowska[1,2]
[1]*Mol Polish Academy of Sciences, 02-247 Warsaw, Poland;* [2]*ReMedy International Research Agenda Unit, 02-247 Warsaw, Poland*

Introduction: Maintenance and turnover of macromolecular complexes constitute a great challenge to the cells, particularly under stress that alters protein homeostasis. Mitochondrial respiratory complexes (OXPHOS) are central machinery responsible for energy production and key elements interconnecting distinct metabolic pathways. We want to understand how cells maintain these elaborate yet damage-prone molecular machines over time and whether they can do it in a sustainable way. We are also curious how the upkeeping and re-purposing of the OXPHOS system entangle in the cell fate decisions and whether intramitochondrial quality control pathways are exploited under disease-associated conditions.

Methods: To get insight into the OXPHOS quality control mechanisms and their pathophysiological relevance, we decided to focus on cancer. Our research employs a set of common breast cancer cell lines and an oncogene-inducible neoplasia model. Hyperproliferation featuring cancer cells imposes strikingly different contexts on the highly organized OXPHOS system by altering the balance between synthesis and degradation of its individual protein components. Meanwhile, repurposing OXPHOS toward anabolic function can associate with different damage patterns. Using a combination of molecular biology, biochemistry and proteomic approaches on parallel cell culture models, we trace OXPHOS assembly/degradation patterns and investigate the underlying mechanisms. **Results and conclusion:** We have previously shown that cells developed dedicated salvage pathway to maintain highly functional respiratory Complex I. This pathway relies on selective proteolytic removal of damaged redox-active parts of the complex mediated by intramitochondrial quality control protease, ClpXP. Loss of ClpXP protease leads to the misbalance in Complex I components inside the mitochondria. Complex I repair is particularly stimulated upon inhibition of the OXPHOS system, but its pathological relevance remains unclear. Our recent results indicate that cancer cells show a highly misbalanced abundance of individual structural subunits of respiratory complexes and a loss of coherency between gene expression and protein levels. Dysregulation of OXPHOS components is accompanied by changes in subunit distribution toward distinct macromolecular assemblies that are not only persistent but also featured by model-specific patterns. Furthermore, our preliminary data suggest that cancer cells can preferentially rely on intramitochondrial proteases and chaperons to ensure appropriate turnover and maintenance of respiratory complexes.

57ASM – 250 | Endothelial IRF3 activation links inflammation and phenotypic changes through metabolic rewiring

J.N. Christensen[1], O. Wagmann[1], J. Blay Cadanet[1], S. Magalhaes Novais[3], P. Bross[2], M. Frye[4], Q. Wu[1], R. Fenton[1], J. Rohlena[3], K. Rohlenova[3], C. Holm[1], J. Kalucka[1,5]

[1]Department of Biomedicine; Aarhus University; Aarhus; Denmark, [2]Research Unit for Molecular Medicine; Aarhus University Hospital; Aarhus; Denmark, [3]Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic; [4]VIB-KU Leuven Center for Cancer Biology, Department of Oncology, KU Leuven, Leuven; Belgium, [5]Aarhus Institute of Advanced Studies (AIAS); Aarhus University, Aarhus, Denmark.

Research objective: Endothelial cells (ECs) play a crucial role in mediating inflammation in (patho)physiological conditions. DNA-induced inflammation contributes to the development of a vast array of diseases, including cancer, infectious and autoimmune diseases. We aimed to investigate the role cGAS-stimulating interferon gene (STING) cytosolic DNA sensing pathway in ECs and the metabolic consequences of endothelial STING activation.
Results: *STING activation alters quiescent endothelial phenotype by inducing inflammation and disrupting cell junctions.*2'3'-cyclic GMP-AMP (cGAMP), activates STING signaling in ECs, which induces the expression of pro-inflammatory cytokines, chemokines and cell adhesion molecules *in vitro* and *in vivo*. Functionally, STING activation leads to decreased cellular migration and angiogenic capacity of ECs due to disruption of Ve-Cadherin junctions and decreased expression of Claudin 5 and Occludin.
STING pathway activation increase OXPHOS.
The phenotypic changes induced by cGAMP-treatment were hypothesized to be associated with EC metabolic rewiring. Targeted metabolomic analysis of cGAMP-treated ECs revealed lower levels of glycolytic intermediates. Transcriptomic and proteomic analyses showed an increased expression of enzymes related to oxidative phosphorylation (OXPHOS). Oxygen consumption rate and Pyruvate dehydrogenase (PDH) activity were increased with STING activation.
STING-induced EC changes are dependent on IRF3
CRISPR-based KO of STING, Interferon Regulatory Factor 3 (IRF3) and Interferon Alpha And Beta Receptor Subunit 1 (IFNAR1) revealed the functional changes are mediated by IRF3 but independent of IFNAR1. Intriguingly, blocking of STING-induced OXPHOS decreased secretion of pro-inflammatory cytokines *in vitro and in vivo*.

Conclusions: STING activation alters EC morphology by inducing junctional and cytoskeletal rearrangement. These alterations decrease the EC capacity to migrate and form vascular sprouts. Mechanistically the alterations depend on IRF3 transcriptional regulation. IRF3 activation results in metabolic rewiring of the endothelium leading to increased OXPHOS. The rewiring is crucial for induction of EC inflammatory responses and EC morphology.

57ASM – 252 | The mechanism of mitochondrial unfolded protein response signaling

Christian Münch
Institute of Biochemistry II, Faculty of Medicine, Goethe University Frankfurt, Germany

The mitochondrial unfolded protein response (UPRmt) is essential to safeguard mitochondria from proteotoxic damage by activating a dedicated transcriptional response in the nucleus to restore proteostasis. Yet, it remains unclear how the information on mitochondria misfolding stress (MMS) is signaled to the nucleus as part of the human UPRmt. I will present new data on how MMS affects mitochondrial and cellular biology to modulate proteostasis networks in the different compartments. We identified that UPRmt signaling is driven by the release of two individual signals in the cytosol –reactive oxygen species and accumulation of mitochondrial protein precursors in the cytosol. These are sensed by the cytosolic HSP70/HSP40 machineries to convey the UPRmt transcriptional response. Our observations reveal a tight link between mitochondrial and cytosolic proteostasis networks that is critical to maintain cellular function.

57ASM – 253 | Targeting mitochondrial potassium channels to eliminate pancreatic adenocarcinoma

M. Bachmann[1]; B. Angi[1]; S. Parrasia[1]; A. Tosi[2]; M. Abdelsalam[1]; A. Mattarei[3]; A. Rosato[2]; A. Carrer[1,4]; E. Gulbins[5]; I. Szabo[1]
[1]*Department of Biology, University of Padova, Italy;* [2]*Department of Oncology and Gastroenterology, University of Padova, Italy;* [3]*Department of Pharmaceutical sciences, University of Padova, Italy;* [4]*Venetian Institute of Molecular Medicine, University of Padova, Italy;* [5]*Department of Molecular Biology, University of Duisburg-Essen, Germany*

Introduction: While great strides have been made in the treatment of many types of cancer, the prognosis for pancreatic ductal adenocarcinoma (PDAC) patients remains very poor, with only 9% of patients surviving more than 5 years. Our teams have recently developed mitochondria-targeted inhibitors that selectively kill cancer cells, by specifically targeting the mitochondrial potassium channels mtKv1.3 and mtKCa3.1. These ion channels are highly expressed in human PDAC cells as well as in specific immune cells of the tumor microenvironment.
Materials and methods: We employed genetic and orthotopic models of PDAC in order to investigate the effect of the above drugs either alone or in combination treatment using chemotherapeutics employed in the clinic. The effect of the drugs was studied both on cancer cells and on immune cells of the tumor microenvironment (TME).
Results*:* The action of the combined treatment, leading to a strong reduction of PDAC was found to be associated with changes of the immune cell populations in the TME and with changes in sphingolipid metabolism in PDAC cells, suggesting that a mitoK channel-sphingolipid axis plays a role in tumor clearance. Importantly, no toxicity was detected upon treatment.
Conclusion: Simultaneous modulation of the behavior of both cancer cells and immune cells in the tumor microenvironment by acting on ion channels of cells' power houses may represent a promising strategy against hard-to-treat cancers.

57ASM – 254 | Nucleus associated mitochondria: a novel signaling conduit and target in cancer

D. Strobbe[1]; D. Faccenda[2,3]; M. Rigon[1,2]; E. Sidlauskaite[2]; B. Kannan[2]; M. Campanella[2,4,5,6]
[1]*Department of Biology, University of Rome TorVergata, 00133 Rome, Italy;* [2]*William Harvey Research Institute, Queen Mary University of London, EC1M 6BQ United Kingdom;* [3]*University of Hertfordshire, Department of Clinical, Pharmaceutical and Biological Science, Hatfield, United Kingdom;* [4]*University College London Consortium for Mitochondrial Research, WC1 0TU Gower Street, London, United Kingdom;* [5]*Institute Gustave Roussy, 94805, France;* [6]*Department of Biomedical Science, University of Padua, 35131, Italy*

Introduction: Loss of mitochondrial quality is one of the hallmarks of cancer. The accumulation of defective mitochondria in transformed cells triggers compensatory pathways which ignite adaptive mechanisms. Recently we uncovered that the mitochondrial retrograde signaling with the nucleus is facilitated by the formation of contact sites which we termed Nucleus Associated Mitochondria (NAM).

Formation of NAM requires remodeling of mitochondria, evasion of mitophagy and stabilization of a subset of molecules such as the 18kDa Translocator Protein (TSPO) via which we trace and modulate this new inter-organellar contact site.

Keen to learn the NAM contribution to cancer progression and therapy evasion we have investigated their role in Glioblastoma Multiforme (GBM): the most common and aggressive type of primary brain tumor.

Methods: We adopted an array of protocols spanning molecular and cellular biochemistry, *in vitro* pharmacology, structural and ultrastructural imaging in GBM patients derived material and cell lines. In these, we profiled core parameters of mitochondrial function and redistribution, quality control processes mediated by autophagy, cell signaling and metabolism. We appraised patterns of transcription factors and susceptibility to demise in response to chemotherapy.

Results: The data collected show that formation of NAM is required for the signaling at the basis of GBM aggressive behavior and resistance to Temozolomide (TMZ). The latter triggers redistribution of mitochondria on the nucleus to pivot, via the formation of cholesterol-rich domains, accumulation and stabilization of nuclear transcriptional factors such as YAP/TAZ and SREBP-1. Molecular and pharmacological repression of NAM counteracts this reducing the proliferative potential of glioblastoma cells and re-instating susceptibility to TMZ.

Conclusions: NAM regulate cell signaling and metabolism and their exploitation lies at the basis of aggressive behavior and therapy evasion of cancer cells. A novel pathogenic mechanism via which improves diagnosis and therapy is therefore proposed.

Keywords: Mitochondria, Retrograde Signaling, Cholesterol, Glioblastoma and Chemotherapy

SYMPOSIUM 2 – CARDIOVASCULAR AND METABOLIC DISEASES

57ASM – 005 | Association of XbaI polymorphism of ApoB gene with risk factors in coronary artery disease Egyptian patients

A. Almenshawy; K. El-Sayed; A. Osman; R. M. El-Farahaty; N. Mahsoub
Faculty of Medicine – Mansoura University, El-mansoura, Egypt

Background: Coronary artery disease (CAD) is a complex cardiac and metabolic disorder. It is thought to be a result of gene–gene and/or gene–environment interactions. It was reported that XbaI polymorphism (rs693) of ApoB gene is associated with CAD. However, the results were conflicting and unclear. Many risk factors were not fully studied in the previous studies.

Aim: It was to assess the association of XbaI polymorphism (rs693) of ApoB gene with lipid profile, risk factors and dietary pattern in CAD Egyptian population.

Subjects and Methods: This study was conducted on 400 Egyptian subjects (200 healthy subjects; 100 Rural and 100 Urban controls and 200 CAD patients; 100 Rural and 100 Urban patients). CAD patients were diagnosed on the bases of clinical, laboratory and angiographic assessment. Polymerase chain reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) was used to genotype ApoB XbaI polymorphism of ApoB gene.

Results: The ApoB XbaI X+X+ genotype frequency was higher in urban control versus urban CAD subgroups. Multivariate analysis revealed that male gender, hypertension, smoking, increased body mass index (BMI), high serum total cholesterol (TC), low serum high density lipoprotein cholesterol (HDL-C) and high score of unhealthy diet were associated with CAD increased risk, while high score of healthy diet was protective.

Conclusion: The genotype X+X+ of ApoB XbaI was significantly associated with CAD prevention in urban subgroup.

57ASM – 041 | Hypoalbuminemia as a 6-month predictor of all-cause mortality among patients hospitalized with acute decompensated heart failure

R. Barbiero[1]; M. Biancucci[2]; F. Dentali[3]; A. M. Maresca[3]; A. Bonaventura[4]
[1]Insubria University, Varese, Italy; [2]Causa Pia Luvini Hospital, Cittiglio, Italy; [3]Department of medicine and surgery, Insubria University, Varese, Italy; [4]Ospedale di Circolo e Fondazione Macchi, ASST Sette Laghi, Varese, Italy

Introduction and Background: Hypoalbuminemia is encountered among elderly patients. In addition, hypoalbuminemia has been described in hospitalized patients with acute decompensated heart failure (ADHF) and associated with reduced survival. We aimed at assessing the clinical impact of hypoalbuminemia among older patients with ADHF admitted to the Internal Medicine unit of a teaching hospital.

Materials and Methods: This interim analysis considered 150 out of 307 patients who are part of a retrospective cohort study of patients consecutively admitted for ADHF

between January and June 2022 to the Internal Medicine unit of Ospedale di Circolo e Fondazione Macchi (ASST Sette Laghi, Varese, Italy). Patients were included among those with discharge ICD-9-CM codes related to ADHF (428.0 to 428.9). The primary endpoint of this analysis was the role of hypoalbuminemia (≤3.5 g/dL) in predicting 6-month all-cause mortality.

Results: Out of 150 patients, 142 had serum albumin levels available (median value 3.2, interquartile range 2.9–3.5 g/dL). The prevalence of hypoalbuminemia was 74% (105/142 patients). No differences were found between patients with hypo- and normoalbuminemia in terms of baseline demographics, comorbidities, and laboratory findings. During the 6-month follow-up, 39 (27.5%) patients died and the survival was significantly lower in patients with hypoalbuminemia compared with those without (33% vs. 11%, log-rank χ^2 6.9, $p = 0.008$; unadjusted hazard ratio [HR] 3.66, 95% confidence interval [CI] 1.23–10.29, $p = 0.014$). After adjusting for multiple, potential clinical confounders (age, sex, hypertension, type 2 diabetes, coronary artery disease, atrial fibrillation, peripheral artery disease, chronic obstructive pulmonary disease, chronic kidney disease, obesity, active cancer, dementia), hypoalbuminemia independently predicted 6-month all-cause mortality (adjusted HR 3.34, 95% CI 1.15–9.66, $p = 0.026$).

Conclusion and Recommendations: Hypoalbuminemia is very frequent among patients admitted to an Internal Medicine unit for ADHF and independently associated with 6-month all-cause mortality.

57ASM – 043 | A deep learning approach to the automatic segmentation of electrocardiograms

F. Raaijmakers[1,2]; M. Vessies[1]; R. van de Leur[1]; D. Schipaanboord[1]; A. Echavarria[1]; M. Schuurbiers[3]; J. ten Broeke[1,3]; R. van Es[1]

[1]UMC Utrecht, Utrecht, Netherlands; [2]University of Amsterdam, Amsterdam, The Netherlands; [3]University of Twente, Enschede, The Netherlands

Introduction: Correct analysis of the electrocardiogram (ECG) allows for diagnosing various cardiovascular diseases. Computer-aided ECG analysis methods were introduced to assist clinicians in the time-consuming task of ECG interpretation. Currently, General Electric's Marquette (the GE-algorithm) is a commonly used algorithm in clinical practice that calculates conduction intervals. We propose a deep learning segmentation model (DLSM) that can extract conduction times with a smaller error than the GE-algorithm.

Aims of the Study: To compare physician annotated conduction intervals with those calculated by the GE-algorithm and our DLSM approach.

Materials and Methods: A database containing 1426 ECGs was used, obtained during routine clinical care in a Dutch academic hospital, physician annotated for on- and offsets of P-, QRS- and T-waves. We randomly split the data 90:10, training and test respectively. The performance was examined by extracting the PQ-, QRS- and QT-intervals of the test set and determining the error by comparing the results to the annotated intervals.

Results: The DLSM was able to extract the PQ-, QRS- and QT-intervals with an average error of 5.2 ± 4.6, 3.8 ± 4.9 and 6.4 ± 12 ms, respectively. GE computes the PQ, QRS and QT-intervals with an average error of 11 ± 23, 9.1 ± 12 and 20 ± 21 ms respectively. In addition, GE failed to detect *p*-waves in five of the 142 test samples. The DLSM error on the PQ-interval was significantly lower than that of the GE algorithm ($p = 0.01$), as well as on the QRS- and QT-intervals ($p < 0.001$).

Conclusion: Our proposed DLSM is able to derive conduction times of PQ-, QRS- and QT-intervals with a significantly smaller error than the currently frequently employed GE algorithm. These results suggest that our DLSM approach is a promising next step in the automatic interpretation of ECGs.

57ASM – 066 | Detecting conduction abnormalities with a four precordial electrodes set-up

J. ten Broeke[1,2]; A. Zepeda Echavarria[1,3]; M. Vessies[1]; M. van der Meer[1]; R. van der Leur[1]; P. Doevendans[1]; J. Jaspers[1,3]; R. van Es[1]

[1]Cardiology Department of the University Medical Centre Utrecht, Utrecht, Netherlands; [2]University of Twente, Enschede, Netherlands; [3]Medical Technology and Clinical Physics Department of the University Medical Centre Utrecht, Utrecht, Netherlands

Background: The miniECG, a smartphone sized portable device capable of recording ECGs with 4 precordial electrodes, is a potential low-cost, easy-to-use alternative for the 12-lead ECG in the hospital and may even facilitate home ECG monitoring. To record an ECG, the device is placed centrally on the chest with the upper two electrodes in the second intercostal space.

Research aim: This study aimed to define criteria to detect left and right bundle branch blocks (LBBB and RBBB) by the miniECG.

Methods: MiniECG recordings were obtained in addition to a clinical 12-lead ECG at the University Medical Centre Utrecht. MiniECG recordings of patients with confirmed BBB on their 12-lead ECG were selected. QRS polarity and morphology and T-wave polarity were determined.

Results: In this preliminary analysis, 40 patients with LBBB (27 male) and 40 patients with RBBB (23 male) were included. In 90% of the patients with LBBB, the miniECG lead measured from the upper left to the lower right electrode, showed negative QRS complexes (in 85% of the patients a broad, deep S-waves that may be preceded by a small r or followed by an S′) and discordant T-waves. In this same lead, positive QRS complexes (in 65% of the patients broad R-waves that may be preceded by a q or followed by an R') were observed in 88% of patients with RBBB.

Conclusion: This study demonstrates that the miniECG is able to differentiate a LBBB from a RBBB. Further research is needed to assess the diagnostic performance of the miniECG to distinguish BBBs from other ECG-abnormalities.

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57ASM – 072 | Response to short nutritional intervention predicts later susceptibility to obesity

K. Bardova; I. Irodenko; J. Funda; K. Adamcová; O. Horáková; P. Janovská; L. Leňková; P. Zouhar; M. Rossmeisl; J. Kopecký

[1]*Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic*

Murine strains differ in their propensity to obesity induced by high-fat diet (HFD) feeding. A/J mice, as compared with C57BL/6J (B6) mice, exhibit lower susceptibility to obesity. Despite the fact that response to acute nutritional intervention might predict later ability of organism to adapt, short-time exposure to HFD in A/J versus B6 mice has not been studied yet. The aim of this study was to detect metabolic flexibility, at both whole-body and tissue level, in murine strains differing in susceptibility to obesity. Male A/J and B6 mice at the age of 8 weeks were exposed to standard (STD) or HFD containing 60% of calories as fat for 3 days. They underwent oral glucose tolerance test, insulin tolerance test, lipid tolerance test or 4-days indirect calorimetry either at 22°C or at 30°C, after 2-week temperature acclimatization. Alternatively, food intake was characterized or mice were euthanized after or during the 3rd day of dietary treatment. Plasma parameters and expression of selected genes were characterized in epididymal white adipose tissue (eWAT). A/J mice after the short-term exposure to HFD as compared to B6 mice displayed healthier metabolic parameters. The circadian rhythms in respiratory quotient and food intake were preserved after 3 days on HFD in A/J mice only. A/J mice also adapted to HFD by an increase in energy expenditure and body temperature both in mice pre-acclimated to laboratory temperature and thermoneutrality. As we did not detect any changes in heat losses through the tail as the main thermoregulatory organ in mice, we assumed higher body-temperature set point. We detected higher expression of genes for leptin in eWAT and higher plasma leptin levels in A/J mice on HFD. Our results document strain-specific difference in metabolic flexibility between A/J and B6 mice during short-term HFD-feeding. These differences predict propensity to obesity.

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57ASM – 086 | Cardiac ischemic tolerance of chronically hypoxic hearts: The role of hypoxia-inducible factor 1alpha and mitochondria

P. Alánová[1]; B. Opletalová[1]; L. Alán[1]; R. Bohuslavová[2]; K. Holzerová[1]; J. Neckář[1]; F. Di Lisa[3]; G. Pavlínková[2]; F. Kolář[1]

[1]*Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic;* [2]*Institute of Biotechnology, Czech Academy of Sciences, Vestec, Czech Republic;* [3]*Department of Biomedical Sciences, University of Padova; National Research Council of Italy (CNR), Padova, Italy*

Transcriptional factor HIF-1α is known to contribute to cardioprotection against ischemia/reperfusion injury. Adaptation to chronic hypoxia is a cardioprotective phenomenon associated with HIF-1α stabilization. However, its precise role in protective changes induced by chronic hypoxia is still not fully understood. The aim of this study was to determine whether partial Hif1a deficiency will abolish the cardioprotective effects of chronic hypoxia as well as its effect on mitochondrial function and dynamics. Adult male wild type and heterozygous Hif1a knockout mice were adapted to chronic hypoxia or kept in normoxia. Physiological responses to chronic hypoxia were assessed and myocardial infarction was induced in isolated perfused hearts. Expression analyses, mitochondrial respiration measurement and electron microscopy were performed to evaluate mitochondrial characteristics. Our results showed decreased infarct size in chronically hypoxic wild type mice compared to their normoxic counterparts. In contrast, this protective effect of chronic hypoxia was absent in mice with partial Hif1a deficiency. Then we showed reduced mitochondrial respiration,

mitochondrial mass together with altered mitochondrial ultrastructure only in chronically hypoxic wild type mice compared to their normoxic control mice. To explain the presence of a higher number of enlarged mitochondria with concomitant reduced mitochondrial mass, we hypothesized that mitophagy might have occurred. We performed microtubule-associated light chain protein 3 assay to monitor autophagy in the presence and absence of lysosomal protease inhibitor leupeptin. The increased autophagosome formation appeared only in chronically hypoxic wild type mice. These findings indicate that HIF-1α regulated mitochondrial processes in cardiac myocytes during adaptation to chronic hypoxia and that HIF-1α was crucial for chronic hypoxia-induced myocardial protection against ischemia/reperfusion injury likely by promoting mitophagy.

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57ASM – 088 | Fat-heart entanglement: The osteopontin mechanics

F. Carbone

University of Genoa, Genoa, Italy

In quantum physic, entanglement identifies a group of particles that interact in a way such that the quantum state of each one cannot be described independently of the state of the others, even when they are far away.

In a similar way, cardiometabolic system is naturally entangled through different types of interactions. The search for hyper-precox markers of cardiometabolic entanglement is an open field of research. For instance, microalbuminuria is likely the earliest sign of cardiometabolic risk since late 90s. We long time focus on osteopontin (OPN) as cardiometabolic mediator with many findings related to different stages and targets.

More recently, we tested OPN as potential hyper-precox marker of cardiometabolic-related injury. In two different cohorts of patients with diabetes alone or clustered within metabolic syndrome, serum and urinary excretion of osteopontin was independently associated with the early clinical signs of diabetic cardiomyopathy or even improvement/regression of metabolic syndrome. Although preliminary and unpowered, our data lean on a growing awareness of OPN as upstream mediator of inflammation, expression of the primum moves of cardiometabolic disease, namely senescence. Here, we briefly discussed the advance of our research and scientific literature on this topic.

57ASM – 090 | Urinary excretion of osteopontin is associated with response to dietary intervention in subjects with Metabolic Syndrome

M. Moriero[1]; M. Bertolotto[1]; D. Verzola[1]; R. Pontremoli[1,2]; L. Pisciotta[1,2]; Francesca Viazzi[1,2]; F. Montecucco[1,2]; F. Carbone[1,2]

[1]*Department of Internal Medicine, University of Genoa, Genoa, Italy;* [2]*IRCCS Ospedale Policlinico San Martino, Genoa, Italy*

Background and Aims: although microalbuminuria is recognized the earliest sign of cardiometabolic risk since late 90s, the search of hyper-precox markers is still ongoing. Here, we focus on urinary excretion of osteopontin (uOPN).

Material and Methods: outpatient subjects with established MetS and enrolled in a validated cohort were retrospectively sub-analysed. All patients received nutritional advice based on Mediterranean-like dietary pattern. Other ongoing medical therapies were maintained. To focus of very early organ damage, patient with microalbuminuria were further excluded and 62 patients were finally included. Primary outcome was testing uOPN as reliable marker of MetS improvement during a 1-year long follow-up.

Results: Intervention generally led to a metabolic improvement in terms of MetS defining criteria ($n = 40$ vs. 22) with a remission rate (<3 defining criteria) of about 44% (p-value <0.001). Such an improvement concerned anthropometric measures: waist circumference ($p = 0.001$), body weight ($p = 0.002$), and body mass index ($p = 0.003$). Meanwhile, metabolic profile improved as well: total cholesterol ($p < 0.001$), low-density lipoprotein cholesterol ($p = 0.003$), triglycerides ($p < 0.001$), fasting glycemia ($p = 0.074$) and glycated haemoglobin ($p = 0.012$) decreased, whereas high-density lipoprotein cholesterol raised ($p < 0.001$). Inflammatory biomarkers (sC-reactive protein, sOPN) and those related to renal impairment (creatinine, uOPN, and OPN-to-creatinine ratio [uOCR]) did not modify during the study with the exception of albumin-to-creatinine-ratio ($p = 0.012$). However, Mets improvement/remission was independently associated with higher baseline values of uOPN in both univariate (OR 7.8 [2.0 to 30.8]) and adjusted model (7.4 [1.9 to 29.9]). This observation was internally validated through bootstrap resampling (OR 7.4 [2.1 to 88.8]) and characterized by relevant discrimination ability (AUC 0.75).

Conclusions: These preliminary data highlight the opportunity to test hyper-precox markers of cardiometabolic risk in primary cardiometabolic prevention. Although unpowered, our data lean on a growing awareness of OPN

as active mediator in cardiometabolic disease and strongly suggest to move on this path.

Keywords: metabolic syndrome, osteopontin, creatinine, inflammation, Mediterranean diet.

57ASM – 095 | Triglyceride-Glucose Index: A surrogate marker of insulin resistance as simple tool to predict long-time diabetes remission after metabolic surgery

C. Sgura[1]; A. Scuricini[1]; G. Adami[1]; L. Liberale[1,2]; F. Saverio Papadia[2,3]; F. Montecucco[1,2]; F. Carbone[1,2]
[1]Department of Internal Medicine, University of Genoa, Genoa, Italy; [2]IRCCS Ospedale Policlinico San Martino, Genoa, Italy; [3]Department of Surgery, University of Genoa, Genoa, Italy

Background and aims: Metabolic surgery remains the most effective strategy for diabetes remission, but long term relapses are not uncommon. Here we focus on triglyceride-glucose (TyG) index as outperforming measure for insulin resistance.

Materials and Methods: Forty obese and diabetic subjects candidates for metabolic surgery and enrolled in a validated cohort were retrospectively sub-analysed. Alongside anthropometric parameters, TyG index was calculated from biochemical panel at baseline and during the first three years of follow-up according with the formula: ln [fasting triglyceride (mg/dL) × fasting glucose (mg/dL)]/2. Testing the predictive value of TyG index variation at 1 year (Δ T0-1y) towards diabetes remission at three year after surgery was set as primary outcome. Characterization of TyG and associations with anthropometric/metabolic changes after surgery were set as secondary ones.

Results: After surgery, anthropometric/metabolic status significantly improved. The rate of diabetic patients also decreased to 45% at 1 year and 67% at 3 year. The extent of TyG index reduction at 1 year was linearly associated with anthropometric measures at baseline and their variations at 1 year as well. In line, both anthropometric values (T0 and Δ T0-1y) and Δ T0-1y TyG index were independently associated with diabetes remission at 1 year (OR 1.26 [1.07 to 1.48]) and 3 years after surgery (OR 1.36 [1.19 to 1.54]). In adjusted models, anthropometric measures (Δ T0-1y weight and BMI) overcome Δ T0-1y TyG index at 1 year but not at 3 years, where it was the only independent predictor of diabetes remission (1.02 [1.01 to 1.50]).

Conclusion: TyG index is a simple and inexpensive marker of insulin resistance with likely higher sensitivity than HOMA-IR index. Although unpowered, our data support the opportunity to further test it and clearly define its clinical use.

57ASM – 099 | The search of hyper-precox markers of diabetic cardiomyopathy: Focus on osteopontin

A. Scuricini[1]; F. Andreozzi[2]; C. Sgura[1]; S. Ministrini[3,4]; M. Bertolotto[1]; L. Liberale[1,5]; G. C. Mannino[2]; E. Succurro[2]; G. Armentaro[2]; V. Cassano[2]; S. Miceli[2]; M. Perticone[2]; F. Montecucco[1,5]; G. Sesti[6]; A. Sciacqua[2]; F. Carbone[1,5]
[1]Department Of Internal Medicine, University Of Genoa, Genoa, Italy; [2]Department of Medical and Surgical Sciences, University "Magna Grœcia"; of Catanzaro, Catanzaro, Italy; [3]Internal Medicine, Angiology and Atherosclerosis, Department of Medicine and Surgery, Università degli Studi di Perugia, Perugia, Italy; [4]Center for Molecular Cardiology, University of Zürich, Schlieren, Switzerland; [5]IRCCS Ospedale Policlinico San Martino, Genoa, Italy; [6]Department of Clinical and Molecular Medicine, University of Rome-Sapienza, Roma, Italy

Background and Aims: Awareness about "diabetic heart" is growing but mainly focused beyond the onset of microalbuminuria. Here we focus on the earliest sign of diabetic cardiomyopathy (DbCM) and the potential role of osteopontin (OPN) hyper-precox marker.

Material and Methods: among outpatient subjects enrolled in the CATAMERI study, those with preserved ejection fraction and without microalbuminuria were selected. Serum OPN was then correlated with echocardiographic parameters and phenotypes/severity of diabetic cardiomyopathy defined by: (i) left atrial dilatation (LAD), (ii) left ventricular hypertrophy (LVH); (iii) presence of diastolic dysfunction (LVDD).

Results: All patients had LAD and the rate of hypertension was 87%. All patients filled 1 (16.3%), 2 (46.1%) or 3 (37.6%) criteria for DbCM. OPN has relevant associations with duration of diabetes (B 7.5 [2.2 to 12.7]) and decline of renal function, (B for eGFR -22.8 [−36.4 to −9.1]). OPN was then tested as independent marker of LVDD (OR 5.3 [1.4 to 20.2]) independently of its abovementioned determinants (B 1.6 [0.1–3.12]). More generally, OPN increase with the number of criteria for DbCM and discriminated the likelihood to have 2 (OR 1.1 [1.0 to 1.2]) or 3 (OR 1.1 [1.0 to 1.2]) of those. Even once dichotomized, (1 vs. 2 and 3 criteria) OPN remained the only associated variable (OR 1.1 [1.0 to 1.2]), also independently of its determinants (B 1.2 [0.1 to 2.2]).

Conclusions: These preliminary data highlight the opportunity to test OPN as hyper-precox markers of DbCM. Although unpowered and biased by the high rate of hypertension and the lack of control group, our data lean on the growing awareness of OPN as active mediator in DbCM and strongly suggest to move on this research field.

57ASM – 113 | Not body size but shape affect progression free survival during treatment with pembrolizumab: Focus on muscle-to-visceral fat ratio

A. Tirandi[1]; A. S. Tagliafico[2,3]; D. Ramoni[1]; S. Coco[3]; G. Rossi[3]; C. Dellepiane[3]; E. Bennicelli[3]; S. Marconi[3]; L. Zinoli[1]; G. Barletta; F. Montecucco[1,3]; C. Genova[1,3]; F. Carbone[1,3]

[1]Department of Internal Medicine, University of Genoa, Genoa, Italy; [2]Department of Health Sciences, University of Genoa, Genoa, Italy, Genoa, Italy; [3]IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Background and Aims: The obesity paradox has long time application in lung cancer but several gaps remain. Here we focus on the abdominal shape and its component: skeletal muscle mass and visceral adipose tissue.

Material and Methods: due to substantial differences in body composition we here considered only male subject with lung cancer candidate to treatment with pembrolizumab, alone or in combination with chemotherapy. At quantitative analysis of the first CT scan performed before treatment starting, abdominal extension of the target tissues was discriminated. Primary outcome was then testing the association between their extension/ratio towards progression free survival (PFS) according with the iRECIST criteria.

Results: Fifty-six male were enrolled. Neither skeletal muscle mass nor visceral adipose tissue alone significantly impact on disease progression, but their ratio did. Having a muscle-to-visceral fat (M/V) ratio below the median value was indeed associated with longer PFS (10 vs. 4 months; p-value = 0.02) and overall survival (14 vs. 9 months; p-value = 0.04). A linear inverse association between M/V ratio and time to progression was also observed (B -0.58 [95%CI: −1.11 to −0.05]), alongside with the expected associations with age, renal impairment and inflammation. Cox-regression analysis confirmed M/V ratio as independent predictor of disease progression, also after adjustment (HR: 4.43 [95% CI 1.41 to 13.94]).

Conclusions: These preliminary data highlight the opportunity to characterize/quantify body shape during CT staging of lung cancer. Although unpowered, our data lean on a growing awareness about the entanglement between cardiometabolic and oncological diseases. Further advance in computational algorithms are expected to improve shape definition and its predictive ability.

Keywords: lung cancer, immunotherapy, computed tomography, progression free survival, skeletal muscle, visceral adipose tissue.

57ASM – 128 | CYP4F2 and VKORC1 Polymorphisms and the Risk of Ischemic Stroke

S. Vesa[1]; S. Ilut[2]; L. Perju-Dumbrava[2]; V. Vacaras[2]; R. Pop[1]; D. Sipos-Lascu[2]; C. Barsan[2]; A. D. Buzoianu[1]; S. Crisan[3]

[1]Department of Pharmacology, Toxicology and Clinical Pharmacology, 'Iuliu Hatieganu' University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]Department of Neurology, 'Iuliu Hatiegan' University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3]Department of Internal Medicine, 'Iuliu Hatieganu' University of Medicine and Pharmacy, Romania

Introduction: The aim of the study was to established if CYP4F2, VKORC1 and GGCX polymorphisms are linked with acute ischemic stroke.

Material and Methods: The patients ($n = 249$) were included from those admitted for acute ischemic stroke at the Department of Neurology from Emergency County Hospital of Cluj-Napoca in the timeframe February 2019–February 2020. The control group included patients ($n = 250$) without acute ischemic stroke admitted at the Departments of Internal Medicine, Cardiology and Geriatric-Gerontology from Municipal Clinical Hospital of Cluj-Napoca. The following data were recorded for each patient: age, gender, living environment, smoking status, obesity (BMI > 30 kg/m2), presence of ischemic heart disease, history of myocardial infarction, arterial hypertension, heart failure, diabetes mellitus or dyslipidemia. Blood lipid profile was also recorded. The presence of carotid plaques was noted. Genotyping of the VKORC1 (G-1639A), CYP4F2 (1347 G > T), and GGCX (12970 C>G) polymorphisms was performed from venous blood.

Results: Stroke patients were more frequently smokers and had arterial hypertension, ischemic heart disease, carotid plaques or a history of myocardial infarction. The VKORC1 (G-1639A) mutant genotype was more frequent in the stroke group. The multivariate logistic model revealed that smoking (OR 3.8; $p < 0.001$) and the presence of carotid plaques (OR 2.4; $p < 0.001$) were independently associated with stroke. The VKORC1 polymorphism m/m genotype (OR 1.9; $p = 0.02$) and the history of MI (OR 3.2; $p = 0.01$) were also linked to the stroke, but after the Bonferroni correction, the p threshold was slightly passed.

Conclusion: Smoking and the presence of carotid plaques were variables independently associated with acute ischemic stroke. The VKORC1 polymorphism might be associated with the risk of ischemic stroke.

57ASM – 131 | The mitochondriotropic antioxidant antiocin4 prevents NAFLD/Nash-induced cardic alterations

C. Magalhães[1]; C. Veloso[1]; R. Silveira[1]; I. Djedovic[1]; V. Mendes[1]; A. Karkucinska-Wieckowska[2]; R. Amorim[1]; I. C. M. Simões[3]; S. Benfeito[3]; B. Manadas[1]; F. Cagide[3]; F. Borges[3]; P. J. Oliveira[1]; M. R. Wieckowski[4]; J. Teixeira[1]
[1]CNC- Center for Neuroscience and Cell Biology, CIBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [2]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland; [3]CIQUP-IMS/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal; [4]Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland

Introduction: Nonalcoholic fatty liver disease (NAFLD) global prevalence is estimated to be about 25%–40%. The first symptomatic manifestations are frequently associated with extra-hepatic complications, such as cardiovascular disease (CVD). NAFLD involves progressive fat deposition, oxidative stress, inflammation and mitochondrial dysfunction. Hence, one approach for treating NAFLD/NASH and associated extra-hepatic complications involves targeting mitochondria oxidative damage in different organs. We previously developed a mitochondriotropic antioxidant (AntiOxCIN4) by conjugating the antioxidant caffeic acid with triphenylphosphonium cation (TPP+).
Materials and Methods: The present work aimed to demonstrate that AntiOxCIN4 (2.5 mg/day/animal) prevents cardiac alterations in a C57BL/6J mice fed with a high-fat (30%), high-sucrose (30%) (HFHS) diet for 16 weeks. In vitro, rat cardiomyoblast cells (H9c2) were treated with AntiOxCIN4 (12.5 μM, 48 h) in the absence/presence of supraphysiologic concentrations of free fatty acids (FFAs) (250 μM, 24 h) for complementary studies.
Results: Histological analysis of cardiac tissue showed that neither HFHS diet nor AntiOxCIN4 induce alterations in structural or inflammatory biomarkers. On the other hand, proteomic analysis showed that HFHS diet induced alterations in the expression of several proteins associated with cardiac metabolism. In vitro, we observed that AntiOxCIN4 increased the levels of protein Troponin-T (cardiac marker), ACOX1 and HADHA (peroxisomal and mitochondrial β-oxidation) and TOMM20 (mitochondrial marker) of H9c2 cells. Therefore, H9c2 cells treated with AntiOxCIN4 showed a more aerobic phenotype.
Conclusion: Our results suggest that HFHS diet primarily induced cardiac metabolic alterations rather than structural changes. Moreover, in vitro, AntiOxCIN4 appears to enhance mitochondrial function, which may contribute to the prevention/treatment of nonalcoholic fatty liver as well as associated cardiac complications.
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57ASM – 133 | NADPH oxidases promote oxidative stress and cardiac dysfunction in the early phase post myocardial infarction

Y. Hao[1,2]; K. Hierdeis[1]; N. Spielmann[3]; Z. Zhang[1]; C. Wolf[4]; A. Görlach[1,2,5]; A. Petry[1]
[1]Experimental and Molecular Paediatric Cardiology, German Heart Center Munich, Munich, Germany; [2]DZHK (German Centre for Cardiovascular Research), partner site Munich, Munich, Germany; [3]Institute of Experimental Genetics and German Mouse Clinic, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany; [4]Department of Paediatric Cardiology and Congenital Heart Defects, German Heart Center Munich, Munich, Germany; [5]Technical University of Munich, School of Medicine, Munich, Germany

Introduction: Coronary artery disease is a leading cause of morbidity and mortality with the most significant consequence being myocardial ischemia. Approximately 25% of patients will not be reperfused thus developing myocardial infarction (MI) and subsequently heart failure with reduced ejection fraction (HFrEF). Oxidative stress has been associated with MI, but its impact on the acute and early response to MI is less well documented. NADPH oxidases have been shown to be major sources of cardiac production of reactive oxygen species. We therefore aimed to characterize the impact of NADPH oxidases on the acute and early phase of MI.
Material and Methods: We generated mice lacking the ubiquitous subunit p22phox required for most NADPH oxidase family members (p22phox-KO) using the Cre/LoxP system. MI was induced by ligation of the left anterior descending coronary artery (LAD). Mice were analysed at days 1 and 7 post-MI. Infarct size was evaluated

by Evans blue and Masson Trichrome staining. Cardiac function was assessed by echocardiography. Oxidative stress was evaluated by 8-hydroxyguanosine (8-OHdG) staining and Western blot for 4-hydroxynonenal (4-HNE). p22phox and F4/80 NA levels were analysed by qPCR.

Results: Oxidative stress markers and p22phox levels were elevated at post-MI days 1 and 7 in hearts from wildtype mice. In hearts from p22phox-KO mice, oxidative stress was prevalent at day 1 post-MI, but was significantly reduced at day 7. Concomitantly, 7 days post-MI, the infarct size size was reduced in p22phox-KO hearts, and echocardiography showed improved ejection fraction and fractional shortening, although expression of the inflammation marker F4/80 remained elevated.

Conclusion: NADPH oxidases are important sources of oxidative stress in the early phase post-MI and promote infarct extension and cardiac dysfunction.

57ASM – 135 | Circulating osteopontin follows closely the natural history of lung cancer subjects treated with pembrolizumab

D. Ramoni[1]; A. Tirandi[1]; C. Genova[1,2]; S. Coco[2]; G. Rossi[2]; C. Dellepiane[2]; E. Bennicelli[2]; S. Marconi[2]; L. Zinoli[2]; G. Barletta[2]; F. Montecucco[1,2]; F. Carbone[1,2]
[1]Department of Internal Medicine, University of Genoa, Genoa, Italy; [2]IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Background and aims: Immune-related adverse events and high cost related to pembrolizumab regimens in advanced non-small cell lung cancer (NSCLC) still remain a caveat. Identifying subjects with greatest expected benefit from pembrolizumab would ideally improve resource allocation. Here we focus on osteopontin (OPN), a pleiotropic protein closely associated with the natural history of lung cancer.

Materials and Methods: Seventy-nine advanced NSCLC candidates for pembrolizumab regimens – alone or in combination with chemotherapy – for first-line therapy of NSCLC were enrolled. Testing baseline serum OPN as predictor of progression-free survival (PFS) according to the iRECIST criteria was set as first outcome. Baseline performance status (ECOG-PS) and overall survival (OS) were set as secondary ones.

Results: Serum OPN progressively with ECOG-PS score ($p = 0.015$) independently of white blood cell/neutrophil count and more specifically than other inflammatory biomarkers (C-reactive protein and matrix metalloproteinase [MMP]-9). A trend to a rise in OPN levels characterized subjects experienced progression of disease/death. Significant was instead the association with time-to-progression (B -2.74 [−4.46 to −1.01]) and time-to death (−0.13 [−0.20 to −0.05]). When Cox regression models were built, OPN showed a substantial prognostic ability towards iRECIST-PFS (HR 6.8 [1.7 to 27.7]) alongside with MMP-9. This model was internally validated through bootstrap resampling (HR 4.0 [1.0 to 14.7]) and characterized by relevant discrimination ability (AUC 0.82).

Conclusion:
Baseline assessment of circulating OPN in serum closely follows clinical presentation and natural history of lung cancer candidate to pembrolizumab regimens in first-line treatment of NSCLC. Although unpowered, our data lean the growing literature about the role of OPN in lung cancer pathophysiology and response to treatments.

Keywords: lung cancer; osteopontin; immunotherapy; pembrolizumab; progression-free survival.

57ASM – 138 | Features and prevalence microvascular dysfunction in patients with coronary artery disease

B. Karimov; A. Abdullaev; J. Uzokov; D. Payziev
Therapy Center, Tashkent, Uzbekistan

Introduction and Background: Microvascular dysfunction is widely discussed and is of great interest in patients with coronary artery disease (CAD). CAD patients with microvascular dysfunction suffer from impaired quality of life more often than not. Aim of the study was to evaluate the features and prevalence of microvascular dysfunction in patients with CAD.

Material and Methods: 232 patients with CAD with the typically angina symptoms who admitted at our Interventional Cardiology department for coronary angiography were assessed (Aged 33–74 years, mean age 52.15 ± 15.92 years, male = 53%). Intracoronary acetylcholine was used coronary functional test for assessment of spasm and microvascular dysfunction. All anthropometry, laboratory and instrumental data were obtained at baseline and results were analysed using by SPSS 26.01 software.

Results: 20.3% (47) of CAD patients had microvascular dysfunction. Patients with microvascular dysfunction had lower use of lipid lowering medications (statins, ezetimibe), renin angiotensin blockers and ranolazine. Abdominal obesity (OR 1.2, CI 95%, 1.04–1.68, $p < 0.05$), dyslipidemia (OR 1.18, CI 95%, 1.05–1.56, $p < 0.05$), female gender (OR 1.15, CI 95%, 1.03–1.78, $p < 0.05$) were associated with microvascular dysfunction in CAD patients. However, smoking, hypertension and symptom severity was not associated with microvascular dysfunction in CAD patients ($p > 0.05$). Coronary artery disease

patients with microvascular dysfunction tended to have higher level of non-HDL cholesterol levels and remnant cholesterol levels than those without it ($p < 0.05$).

Conclusions and Recommendations: Microvascular dysfunction is high among coronary artery disease patients who admitted for coronary angiography due to angina pectoris sympto Female with high abdominal obesity and impaired non-HDL and remnant cholesterols were high risk for the microvascular dysfunction.

57ASM – 139 | Cardiac cachexia is associated with altered expression of genes related to iron and lipid metabolism

P. Janovska[1]; T. Kobets[1]; O. Kuda[1]; L. Steiner-Azova[1,2]; V. Stranecky[2]; V. Melenovsky[3]; J. Kopecky[1]
[1]Institute Of Physiology Of The Czech Academy Of Sciences, Prague, Czech Republic; [2]Department of Paediatrics and Adolescent Medicine, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Department of Cardiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Cardiac cachexia is characterized by loss of fat and muscle mass and develops in about 10% of patients with heart failure (HF). Cachexia is associated with a poor prognosis and cannot be reversed by nutrition. Causes of wasting are poorly characterized. In our previous study, the changes in both metabolome/lipidome and expression of selected genes measured in samples of epicardial adipose tissue (EAT) of patients undergoing heart transplantation indicated dysregulation of EAT lipid metabolism in cachexia. Therefore, the aim of study was to characterize whole transcriptome of both EAT and left ventricle myocardium (MYO) in body weight (BW)-stable ($n = 9$) and cachectic patients ($n = 9$) selected from the previous cohort of HF patients (1) using RNA sequencing. Hierarchical clustering of variabilities in expression of 37 genes, which were differentially expressed in both MYO and EAT, showed high uniformity in expression pattern within both groups, regardless on tissue, depicting strong differences between the phenotypes. The most pronounced pattern in both tissues, and bigger changes in EAT, were found in expression of the genes for stearoyl-CoA desaturase involved in fatty acid synthesis, transferrin involved in iron transport, and proteoglycan 4 counteracting fibrose adhesion and inflammation. Functional annotation revealed terms related mainly to the remodelling tissues, immune system in the BW-stable, and fatty acid and iron metabolism in the cachectic patients. Deeper lipidomic analysis in EAT uncovered potential higher oxidation of polyunsaturated lipids in cachexia. Analysis of significantly regulated phosphatidyl ethanolmanines revealed higher content of arachidonic acid at sn2 position. Changes in iron and lipid metabolism in both EAT and MYO can be key markers of cardiac cachexia, they are more pronounced in EAT, and suggest the role of EAT-MYO microenvironment in the development of HF.

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57ASM – 142 | Epitranscriptomic regulatory mechanisms, cardiomyocyte physiology, and nonconventional cardioprotective interventions

M. Hlavackova[1]; D. Benak[1]; D. Sotakova-Kasparova[1]; K. Holzerova[1]; J. Hrdlicka[1]; P. Telensky[2,3]; A. Simonova[4]; H. Cahova[4]; Al. Pecinova[5]; A. Eckhardt[6]; M. Olsen[7]; T. Kobets[8]; J. Neckar[1]; F. Kolar[1]
[1]Laboratory of Developmental Cardiology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic; [2]Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic; [3]International Clinical Research Center of St. Anne's University Hospital Brno, Dementia Research Group, Brno, Czech Republic; [4]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic; [5]Laboratory of Bioenergetics, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic; [6]Laboratory of Translational Metabolism, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic; [7]Department of Pharmaceutical Sciences, College of Pharmacy-Glendale, Midwestern University, Glendale, United States; [8]Laboratory of Metabolomics, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Introduction: Ischemic heart disease is the leading cause of death worldwide. Cardiac tolerance to ischemia can be increased by cardioprotective interventions such as adaptation to chronic hypoxia or fasting, which are associated with significant myocardial gene expression profile changes. Among the possible mechanisms of gene expression alterations are epigenetic modifications of RNA – epitranscriptomics. We focused on two of the most prominent marks – N6-methyladenosine (m6A) and N6,2′-O- dimethyladenosine (m6Am) and their regulators in the myocardium of chronically hypoxic and fasting rats.

Methods: Adult male rats were adapted to continuous normobaric hypoxia (CNH; 10% O2; 3 weeks) or kept under normoxic conditions. Concerning fasting, adult male rats were kept without a food supply for 3 days. The

control group was fed ad libitum. The levels of m6A/m6Am were measured by LC/MS analysis and colorimetric quantification. Metabolic rates were measured using a Seahorse XFe24 analyser. LC/MS-based metabolomics and DIA-MS-based proteomics were applied to analyse the effect of FTO inhibitor.

Results: We found that in adult rat cardiomyocytes the level of m6Am modification was significantly higher than m6A in rRNA-depleted RNA, which points to the significance of m6Am modification, and FTO as its demethylase. The effect of cardioprotective interventions on the levels of m6A and m6Am regulators in the heart differed. While both adaptation of rats to chronic hypoxia and fasting upregulated the two demethylases (ALKBH5, FTO), the m6A readers (YTHDF1-3) were regulated in opposite directions by these interventions. Inhibition of FTO in control cardiomyocytes increased metabolic rates and affect cellular processes such as nucleotide metabolism, autophagy, hexosamine biosynthetic pathway, and RNA processing. Inhibition of FTO in oxygen-deprived cardiomyocytes decreased their viability and stimulated the mTOR pathway.

Conclusion: Our data show the important role of FTO in cardiomyocyte physiology regulation and support the potential involvement of epitranscriptomic regulations in the cardioprotective mechanisms.

57ASM – 146 | Synergistic Effects of Maternal Diabetes and HIF-1α Dysregulation on Embryonic Development of Cardiac Sympathetic Innervation

P. Hrabalova[1,2]; R. Bohuslavova[1]; V. Fabriciova[1,2]; D. Sedmera[3,4]; G. Pavlinkova[1]

[1]Laboratory of Molecular Pathogenetics, Institute Of Biotechnology Of The Czech Academy Of Sciences, Vestec, Czechia; [2]Faculty of Science, Charles University, Prague, Czechia; [3]Institute of Physiology CAS, Prague, Czechia; [4]Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czechia

Introduction: Maternal diabetes during embryonic development has been associated with adverse health outcomes, including an increased risk of obesity, metabolic dysfunction, and cardiovascular diseases. Cardiac sympathetic innervation plays a critical role in regulating heart function, and any alternation in its development or function may lead to a range of health issues, from sudden infant death syndrome to common adult diseases such as hypertension or heart failure.

Methods: In this study, we used a transgenic mouse model with a conditional deletion of Hif1a (Hif1aCKO) in sympathetic neurons, in combination with our established model of maternal diabetes induced by streptozocin.

Results: Our preliminary data showed that total sympathetic chain size was significantly smaller in diabetic Hif1aCKO embryos compared to the controls. Hif1aCKO hearts developing under the influence of maternal diabetes showed impaired sympathetic innervation. Diabetic environment effected the branching and thickness of innervation, together with the density of sympathetic neurons. Furthermore, the size of the adrenal gland and the number of chromaffin cells in the adrenal medulla was decreased in diabetic Hif1aCKO embryos. RNA sequencing revealed changes between the transcriptome of sympathetic neurons from mutant and control embryos.

Conclusions: Our study indicate that maternal diabetes and Hif1a deficiency have combinatorial effects on the development of the cardiac sympathetic nervous system. Consequently, HIF1a dysregulation may contribute to various sympathetic abnormalities underlying cardiac pathologies, including heart failure and sudden cardiac death. These findings highlight the importance of considering maternal health and genetic factors in the development of adverse cardiac outcomes in offspring.

57ASM – 155 | Investigating the quality of attribution methods on ECG classification

M. Vessies; R. R. van der leur; P. A. Doevendans; R. van Es

UMC Utrecht, Utrecht, Netherlands

Introduction: Interpretation of electrocardiograms (ECGs) is time-consuming and requires extensive training. Deep neural network (DNN) based methods have been proposed to assist the physician in diagnosing ECGs. Several of these algorithms are validated using explainability techniques in the form of attribution methods (often visualized as heat maps) that aim to improve trust among physicians and reduce potential biases. We, however, argue that commonly used attribution -based techniques are too inconsistent to be trusted for medical applications. In this study we compare various attribution methods applied to ECG classification and show their consistency under different settings.

Methods: We trained a binary classification model for the detection of atrial fibrillation (AF) and repeated this for 3 random model initializations. Each tested model had a c-statistic of more than 0.95, in line with current state of the art models. We then assessed the quality of each attribution method in the following ways: 1. Correlation between different attribution methods applied to the same model. 2. Correlation between the same attribution method applied to the same model trained with different initializations.

These checks are used to investigate the distinctiveness and consistency of the attribution methods.

Results: We show that for sanity check 1, the methods show low correlation (<0.2). Check 2 shows mixed results, some methods show consistent results over seeds (maximum correlation of 0.8) while others provide different attributions for each model (minimum correlation of 0.05).

Conclusions: The experiments show little agreement between attribution methods and that it is hard to assess if what the attribution method is showing is what the model is actually looking at. The results from this study confirm our hypothesis that there is too large variation between and within the tested attribution methods to make them clinically applicable. Further research is required to understand the underlying causes.

57ASM – 164 | Polyphenol-Enriched Ethanolic Extract of Sarcopoterium spinosum fruits and coriligin as single agent: Anti-inflammatory and antioxidant effects on endothelial cells

H. Zbeeb[1]; H. Khalifeh[2]; F. Baldini[3]; L. Zeaiter[3]; M. Khalil[4]; L. Vergani[1]

[1]University Of Genova, Genova, Italy; [2]University of Camerino, Camerino, Italy; [3]Istituto Italiano di Tecnologia, Genova, Italy; [4]University of Bari Aldo Moro, Bari, Italy

Introduction: Oxidative stress is underling in the development of many metabolic disorders such as cardiovascular disease, type 2 diabetes mellitus and fatty liver diseases. In physiological conditions, reactive oxygen species (ROS) are continually produced as the consequence of metabolism and balanced by the antioxidant defence system Moreover, ROS regulate vascular cell proliferation, and oxidative stress could lead to hypertension and apoptosis and promote pathological process. The ability of polyphenols in preventing oxidative stress and ameliorating chronic diseases has been proved since long time. Sarcopoterium spinosum, an eastern Mediterranean shrub belonging to the Rosaceae family, is rich in polyphenols. Traditionally, both fruits and roots of S. spinosum are used in herbal medicine, but fruits are poorly investigated in scientific studies.

Aim: To investigate the in vitro antioxidant and cytoprotective properties of an ethanolic extract from S. spinosum fruits (SEE) and corilagin, an ellagitannin identified in the extract, as comparison, using endothelial cells (HECV).

Materials and Methods: H2O2-insulted HECV cells mimic endothelium dysfunction and oxidative stress condition. The beneficial effects of 24 h treatment with either SSE or corilagin after oxidative insult were assessed by measuring the ROS generation, the MDA production, the reduced/oxidized glutathione ratio, and the wound repair.

Results: The preliminary results showed that both the SSE and corilagin significantly ameliorated the oxidative stress condition, and at the same time, they were able to accelerate the wound repair of HECV cells.

Conclusion: The ethanolic extract from S. spinosum fruits, possibly depending on the high content of corilagin, could be a potential candidate for nutraceutical applications.

57ASM – 176 | Intravenous statin administration exerts cardioprotective effects in the setting of myocardial infarction in the presence of diabetic cardiomyopathy

S. Alcover[1]; S. López[1]; A. Gallinat[1]; M. Borrell[1]; L. Badimon[1,2,3]; G. Vilahur[1,2]

[1]Cardiovascular Program, Institut de Recerca, Hospital de la Santa Creu i Sant Pau, IIB Sant Pau, Barcelona, Spain; [2]CiberCV, Institute of Health Carlos III, Madrid, Spain; [3]Cardiovascular Research Chair, UAB, Barcelona, Spain

Introduction: Diabetic patients may develop diabetic cardiomyopathy (DCM) and are at increased risk of myocardial infarction (MI). Yet, cardioprotection remains a challenge in the presence of comorbidities.

Objectives: We examined whether intravenous administration of atorvastatin during ongoing myocardial ischemia prevents against the deleterious effects of MI in DCM rats.

Methods: DCM was induced by streptozotocin in Sprague–Dawley rats ($n = 14$). A normoglycemic-control group (NC) was also run for control purposes ($n = 14$). MI was induced in all animals by transient coronary ligation of the LAD (45 min). Early after ischemia induction (15 min) DCM- and NC- rats were distributed to either receive i.v atorvastatin or vehicle. Animals were reperfused and sacrificed 24 h post-MI for area-at-risk and infarct size assessment and molecular analysis on the infarcted heart. Cardiac function was monitored by echocardiography.

Results: No differences were detected as per the area-at-risk between all animal groups. Intravenous atorvastatin exerted comparable infarct-sparing effects in DMC (32% reduction) and NC (40% reduction) infarcted rats as compared to their respective vehicle-administered groups ($p < 0.05$). Likewise, left ventricle systolic fraction was better preserved in atorvastatin-treated animals as compared to vehicle administered rats (DCM: $-4.6 \pm 2.5\%$ vs. $-12.4 \pm 3.1\%$; NC: $-9.5 \pm 7.9\%$ vs. $-32.8 \pm 11.1\%$) At a molecular level, administration of iv atorvastatin to both

DCM and NC rats was associated with myocardial RhoA inhibition, higher p-AMPK activation and lower apoptosis execution assessed by confocal microscopy ($p < 0.05$ vs. vehicle) in the infarcted heart. In addition, DCM treated-animals showed a significant reduction in neutrophil and macrophage myocardial infiltration in the ischemic heart ($p < 0.05$ vs. vehicle and NCs).

Conclusion: Intravenous administration of atorvastatin during MI limits infarct size and preserves cardiac function post-MI despite the presence of comorbid conditions. These benefits are associated with an enhanced AMPK activation, lower inflammatory cell recruitment and reduced apoptosis execution in the infarcted heart.

57ASM – 193 | The diagnostic value of ECG characteristics for vasospastic and microvascular angina: A systematic review

D. Schipaanboord[1]; J. Woudstra[2]; Y. Appelman[2]; S. Rittersma[3]; T. P. Van de Hoef[3]; R. Van Es[3]; R. Coronel[4]; P. Van der Harst[3]; C. Onland-Moret[5]; H. M. Den Ruijter[1]

[1]Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands; [2]Amsterdam UMC, Heart Centre, Department of Cardiology, Amsterdam Cardiovascular Sciences, Amsterdam, The Netherlands; [3]Department of Cardiology, Division Heart and Lungs, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands; [4]Department of Experimental Cardiology, Amsterdam Cardiovascular Sciences, University of Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands; [5]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

Introduction: Coronary vascular dysfunction comprises vasospastic angina (VSA) and/or microvascular angina (MVA) and is more common in women than in men with angina without obstructive coronary artery disease (ANOCA). Invasive coronary function testing is considered the reference test for diagnosis, but its burden on patients is large. Identification of a non-invasive diagnostic test for coronary vascular dysfunction is therefore important.

Aim: We aimed to investigate the potential of electrocardiography (ECG) as non-invasive marker for VSA and MVA diagnosis.

Methods: We systematically screened Pubmed and EMBASE databases for studies reporting on ECG characteristics in ANOCA patients with (a suspicion of) coronary vascular dysfunction and assessed study quality using QUADAS-2. We extracted data on diagnostic values of different ECG characteristics and analysed whether the studies were sex-stratified.

Results: Thirty publications met our criteria, of which 13 reported on VSA and 17 on MVA. The majority addressed repolarization-related ECG parameters. Only 1 out of 13 VSA papers and 4 out of 17 MVA papers showed diagnostic accuracy measures of the ECG characteristics. The presence of early repolarization, T-wave alternans or inverted U-waves were most promising for VSA diagnosis. The presence was 29–36%, 44%–60% and 79%–89% in VSA patients, respectively, and 6%–12%, 0%–11% and 0%–4%, respectively, in non-VSA patients or controls. The QTc interval was predictive for MVA diagnosis in all 6 studies reporting on QTc interval (mean/median prolongation ranging between 4–30 ms, $p < 0.05$ in all studies). Sex-stratified results were described in only 5 out of 30 studies and 3 of those observed sex-based differences.

Conclusions: ECG features are not widely evaluated in diagnostic studies for VSA and MVA. Those features predictive for VSA and MVA diagnosis mostly point to repolarization abnormalities and may contribute to non-invasive risk stratification.

57ASM – 198 | Endothelial dynamics of DJ-1 and its role in the regulation of ectopic ATP-synthase activity during acute ischemia and reperfusion

A. Gallinat[1]; L. Badimon[1,2,3]

[1]Cardiovascular Program-ICCC, Research Institute-Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, Barcelona, Spain; [2]CIBERCV-Instituto de Salud Carlos III, Madrid, Spain; [3]UAB-Chair Cardiovascular Research, Barcelona, Spain

Introduction: Endothelial cells (ECs) are crucial for the organ response to ischemia. The ectopic expression of ATP-Synthase on the cell surface is now recognized for many cell types, and presumably plays a role in the regulation of intracellular pH and angiogenesis. DJ-1 is a multifunctional protein that regulates mitochondrial ATP-synthase, and is involved in cell protection against ischemia and oxidative stress.

Aim: To investigate the endothelial dynamics of DJ-1 and its role in the regulation of ectopic ATP-synthase (ecATP-S) activity during acute ischemia and reperfusion in ECs.

Methods: Human umbilical vein endothelial cells (HUVECs) were used in this study. Cell cultures were subjected to in vitro ischemia or ischemia–reperfusion (I/R), and both the intracellular and extracellular contents of DJ-1 were measured by western blot. The ecATP-S activity was assessed in normoxia and ischemia in DJ-1 knock-down and wild-type cultures, in the presence and

the absence of 100 nM recombinant DJ-1. Physical interaction between DJ-1 and ecATP-S was evaluated by co-immunoprecipitation. Effects on the angiogenic potential were assessed using the tube-formation assay at reperfusion.

Results: We found that DJ-1 is secreted from ECs, by a mechanism enhanced in ischemia and I/R. A cleaved form of DJ-1 (DJ-1ΔC) was found only in the secretome of ischemic cells. ecATP-S activity increased following acute ischemia in ECs, coinciding with DJ-1 and DJ-1ΔC secretion. DJ-1 knock-down inhibited the ecATP-S response to ischemia by a 50%, while administration of recombinant DJ-1 of either form maximized the effect, enhanced Akt phosphorylation and promoted angiotube-formation at reperfusion. Immunoprecipitation studies corroborated direct interaction between DJ-1 and ecATP-S.

Conclusions: DJ-1 is secreted from ischemic ECs and plays an important role regulating the ecATP-S activity during ischemia and reperfusion. These findings highlight DJ-1 as a potential therapeutic target for ischemia.

57ASM – 202 | Moderate short term cold exposure possesses infarct-size limiting effect

P. Kašík[1]; A. Marvanova[1]; B. Elsnicova[1]; F. Galatik[1]; D. Hornikova[1]; P. Vebr[1]; V. Cigan-Tibenska[1]; P. Vodicka[2]; L. Hejnova[1]; V. Zvolska[1]; B. Szeiffova-Bacova[3]; M. Sykora[3]; J. Novotny[1]; J. Neuzil[1,4,5]; F. Kolar[6]; O. Novakova[1,6]; J. M. Zurmanova[1]

[1]Charles University, Faculty of Science, Department of Physiology, Prague, Czech Republic; [2]Czech Academy of Sciences, Institute of Animal Physiology and Genetics, Libechov, Czech republic; [3]Slovak Academy of Sciences, Institute for Heart Research, Bratislava, Slovakia; [4]Griffith University, School of Pharmacy and Medical Science, Southport, Australia; [5]Czech Academy of Sciences Institute of Biotechnology, Prague, Czech republic; [6]Czech Academy of Sciences, Institute of Physiology, Prague, Czech republic

Introduction: We are searching innovative methods to enhance endogenous signalling pathways that safeguard or enhance cardiovascular health. Our latest research has unveiled an unconventional approach involving short term exposure to mild cold, which improves the heart's resilience against ischemia and reperfusion injury, without inducing hypertension or myocardial hypertrophy. Based on this results we aimed to find the shortest period inducing the cardioprotection.

Materials and Methods: Adult male rats were acclimated to 9°C for varying periods of 1, 3, and 10 days. Our study analysed the left ventricle and brown adipose tissue using a western blotting, ELISA, receptor binding assay, quantitative immunofluorescence microscopy, and oxygraphy. Additionally, we employed a MultiPlex analysis of cytokines in the blood serum to obtain a comprehensive understanding of the results.

Results: We found that the short term cold acclimation reduced the infarct size and affected the resistance of cardiac mitochondria to Ca2+ overload. All three isotypes of β-adrenergic receptors were altered. The changes in oxidative stress was assessed by malondialdehyde assay and selected cytosolic and mitochondrial antioxidant enzymes expression. Energy status was defined by AMP activated kinase and hexokinase expression and localization. Cold-elicited immune modulation was observed on the cytokine expression level.

Conclusion: Our research findings indicate that short term moderate cold exposure possesses cardioprotective effect, while simultaneously affecting mitochondrial function, altering the β1/3-adrenergic receptors and modulating the inflammatory response. Understanding the precise mechanisms underlying this protective effect could be crucial for the successful translation of this approach into preventive or therapeutic strategies for patients with cardiovascular diseases.

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57ASM – 203 | Reinforcement of β-adrenoreceptor signalling contributes to the chronic mild cold elicited cardioprotection

F. Galatík[1]; A. Žbírková[1]; A. Marvanová[1]; P. Vebr[1]; P. Kašík[1]; V. Tibenská[1]; B. Elsnicová[1]; D. Horníková[1]; M. Vecka[2]; B. Szeiffová Bačová[3]; N. Tribulová[3]; J. Novotný[1]; F. Kolář[4]; O. Nováková[1,4]; J. Žurmanová[1]

[1] Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic; [2]4th Department of Internal Medicine, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia; [4]Institute of Physiology, Czech Academy of Sciences, Prague, Czech republic

Introduction: The effect of cold on the cardiovascular system depends on its severity and duration. Sudden exposure to extreme cold in humans is associated with adrenergic hyperactivation (cold shock) but prolonged exposure to mild cold causes β1-adrenergic receptor (β1-AR) desensitization. We revealed that 5 weeks of mild cold acclimation ameliorates myocardial infarction in vivo which persists for another 2 weeks after acclimation without side

effects such as myocardial hypertension or hypertrophy [1, 2]. We aimed to elucidate the underlying mechanism.
Materials and Methods: Male Wistar rats were exposed to gradual cold acclimation (9 ± 1°C, 5 weeks), while the recovery group was kept at 24°C for additional 2 weeks after acclimation. Myocardial infarction was induced by coronary occlusion for 20 min followed by 3 h reperfusion. We investigated the effect of β1/β2/β3-AR inhibitor administration on the myocardial infarction limiting effect as well as other possible facets of cold elicited cardioprotection.
Results: While the β1-AR inhibitor (metoprolol) administration before ischemia had no effect, and β2-AR inhibitor (ICI-118551) affected only recovery group, the β3-AR inhibitor (SR52930A) affected cardioprotection in both groups. In line with the myocardial infarction limiting effect, mitochondrial resistance to Ca^2+-overload was altered in both groups. Arrhythmogenesis was also affected after cold acclimation and was associated with change in n-3/n-6 polyunsaturated fatty acid ratio and "end to end" orientation of connexin-43 in cardiomyocytes.
Conclusion: Chronic mild cold exposure has a myocardial infarction limiting effect mediated via the alteration of β-AR signalling, persisting for at least two weeks. These results improve our knowledge of mechanisms occurring during cold acclimation and its potential use in prevention and therapy of cardiovascular diseases.
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57ASM – 211 | Influence of low glycemic index diet on short-term clinical outcomes in patients with atherosclerotic coronary artery disease after PCI

D. Payziev; B. Alyavi; J. Uzokov; A. Abdullaev
Republican Specialized Scientific Practical Medical Center of Therapy and Medical Rehabilitation, Tashkent, Uzbekistan

Introduction: Accumulated data suggest that low glycemic index diet (LGID) improved several clinical and biochemical parameters in patients with atherosclerotic coronary artery disease (ASCAD) after percutaneous coronary interventions (PCI) [1]. Aim of the present study was to evaluate the influence of LGID on short-term clinical outcomes in patients with ASCAD.
Material and Methods: The prospective, open label randomized study included 160 ASCAD patients collected within 2016–2019 years (Aged 38–76 years, mean age 58.2 ± 12.0 years, male = 48%). Patients were divided into 2 groups by 80 and assigned either LGID or routine recommended diet (RD). Patients were followed-up to three years after the PCI followed by stenting (medium 18.22 ± 6.54 months) for the assessment of major adverse cardiac events (MACE). All data analysed using STATA software.
Results: At the follow up period mortality rate was higher in RD groups than LGID group (RR1.34, CI 95%, 1.025–1.85, $p < 0.05$). MACE were significantly higher in Group II than Group I patients (RR 1.28, CI 95%, 1.018–1.76, $p < 0.05$). During the follow up 7% of patients occurred ACS whereas 11% patients in RD group occurred ACS ($p < 0.05$). Regarding the stent thrombosis, there were not observed statistically significant changes between groups ($p > 0.05$). Target vessel revascularization were performed in 2.5% LGID group patients whilst 6.25% in RD group patients ($p < 0.05$). Besides, target lesion revascularization was performed in 1.25% LGID patients whilst 3.75% RD group patients ($p < 0.05$). When we analysed clinical outcomes by gender there were not observed any statistically significant changed between men and women ($p > 0.05$).
Conclusion: Low glycemic index diet had lesser risk of MACE than routine diet in the short-term period in patients with atherosclerotic coronary artery disease after PCI. Further studies are required with large amount of patients.

57ASM – 215 | Relationship between blood inflammation state and platelet aggregation rate in patients with atherosclerotic coronary artery disease after percutaneous coronary interventions

O. Mukhitdinova[1]; B. Alyavi[1]; J. Uzokov[2]; D. Orziev[2]
[1]Tashkent Paediatric Medical Institute, Tashkent, Uzbekistan; [2]Republican Specialized Scientific Practical Medical Center of Therapy and Medical Rehabilitation, Tashkent, Uzbekistan

Introduction: Pro-inflammatory and anti-inflammatory interleukins play crucial role for the progression of atherosclerotic coronary artery disease. Aim of the study was to investigate the possible links between blood inflammation state parameters and platelet aggregation rate in patients with atherosclerotic coronary artery disease after percutaneous coronary interventions.
Material and Methods: 110 patients who were diagnosed with atherosclerotic coronary artery disease and planned for elective PCI were enrolled in this study from 2017 to 2021 years (mean age 59.8 ± 8.06 years, male = 86%). Interleukin-1 (IL-1), IL4, IL-6, IL-10, high sensitive C reactive protein (hsCRP) and systemic immune inflammatory index (SII) were assessed for the assessment of

inflammation state. Platelet aggregation rate was analysed with 5 μmoLL ADP on aggregometer before PCI. Pearsons's correlation was used to analyse possible links between inflammatory state and platelet aggregation rate with 5 μmoLL ADP.

Results: After adjusted all confounders there were a positive correlation between IL-1 and 5 μmoLL ADP induced platelet aggregation rate (%) ($r = 0.38$, CI 95%, $p < 0.05$) as well as IL-4 and 5 μmoLL ADP induced platelet aggregation rate (%) (0.45, CI 95%, $p < 0.05$). Even though, there was positive correlation between IL-6 and 5 μmoLL ADP induced platelet aggregation rate (0.25, CI 95%, $p > 0.05$), this correlation were not statistically significant. When we analysed IL-10 and 5 μmoLL ADP induced platelet aggregation rate, there was negative correlation between them ($r = -0.61$, CI 95%, $p < 0.0001$). Regarding the hsCRP there was a slight statistically insignificant positive correlation with 5 μmoLL ADP induced platelet aggregation rate ($r = 0.27$, $p > 0.05$). When it comes to SII, it was strong positively correlated with 5 μmoLL ADP induced platelet aggregation rate (%) ($r = 0.51$, CI 95%, $p < 0.05$). There were not observed any statistically significant changes between sexes.

Conclusion: Inflammation state is over activated in atherosclerotic coronary artery disease patients with increased platelet aggregation rate.

57ASM – 223 | Renal denervation improved right ventricular function and restored norepinephrine levels in rat model of heart failure induced by volume overload

M. Miklovic[1,2]; O. Gawrys[1]; P. Kala[1,3]; Z. Honetschlagerova[1]; S. Jichova[1]; Z. Vanourkova[1]; Z. Huskova[1]; S. Kikerlova[1]; H. Maxova[2]; A. Kvasilova[4]; G. Puertas-Frias[5]; D. Sedmera[4]; T. Acek[5]; V. Melenovsky[6]

[1]Institute of Clinical and Experimental Medicine, Prague, Czech Republic; [2]Department of Pathophysiology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Department of Cardiology, Motol University Hospital, Prague, Czech Republic; [4]Institute of Anatomy, Charles University, Prague, Czech Republic; [5]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic; [6]Department of Cardiology, IKEM, Prague, Czech Republic

Introduction: Heart failure (HF) is accompanied by depletion of myocardial norepinephrine (NE) and increased plasma spillover. Renal denervation (RDN) lowers blood pressure, but its effects on right ventricular (RV) and cardiac sympathetic nervous system function in HF are poorly understood.

Aims: The objective was to investigate the impact of HF and RDN on RV function, myocardial norepinephrine (NE) levels and on molecular pathways responsible for NE metabolism in a volume-overload HF model.

Methods: HF was induced by aorto-caval fistula (ACF) in Ren-2 transgenic rats (TGR) of 8 weeks (w). Bilateral RDN was performed chemically (phenol application) 1w later. 2w after RDN, pressure-volume analysis was performed for RV function assessment and cardiac tissue was harvested. NE was measured by solid phase ELISA kit, protein expression by western blot, gene expression by Taqman PCR gene expression assay and ROS was measured by oxidation of Amplex Red assay.

Results: ACF animals had increased RV systolic pressure, decreased RV function (end-systolic elastance), increased NE in kidney and plasma, but depleted NE levels in RV compared to the sham. ACF rats had also decreased RV protein expression of tyrosine hydroxylase, decreased organic transporter 3 (postsynaptic NE transport), increased protein expression and activity of monoamine oxidase A (MAO-A). RDN in the ACF group improved RV function, decreased RV systolic pressure, decreased NE levels in plasma and kidney, increased protein expression of tyrosine hydroxylase and increased and normalized NE levels in RV.

Conclusion: ACF leads to RV dysfunction and myocardial NE depletion due to increased NE catabolism and impaired reuptake. RDN improved RV function, decreased RV systolic pressure, normalized NE levels and increased tyrosine hydroxylase. We suggest that beneficial effect of RDN on RV in volume overloaded HF is likely due to reduction of sympathetic drive.

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57ASM – 251 | SGLT2 inhibitors and cardioprotection

S. Chen[1]; Q. Wang[1]; A. Christodoulou[2]; N. Mylonos[2]; D. Bakker[1]; V. Wakker[3]; M.W. Hollmann[1]; N.C. Weber[1]; R. Coronel[4]; V. Christoffels[3]; I. Andreadou[2]; C.J. Zuurbier[1]
[1]Laboratory of Experimental Intensive Care and Anesthesiology, Department of Anesthesiology, Amsterdam Cardiovascular Sciences, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands; [2]Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15771, Athens, Greece; [3]Department of Medical Biology, Amsterdam Cardiovascular Sciences, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands; [4]Department of Experimental Cardiology, Amsterdam Cardiovascular Sciences, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

Background: SGLT2 inhibitors now belong to the first-line armamentaria for treatment of chronic heart failure in diabetic and non-diabetic patients. SGLT2 inhibitors are also beneficial in the setting of acute heart failure and in preclinical models of acute myocardial infarction. Ambiguity exists to what extent the beneficial effects of SGLT2is are due to inhibition of the SGLT2 or to non-SGLT2, off-target mechanisms. Employing newly created whole-body SGLT2 KO mice, we hypothesized that (1) chronic deletion of SGLT2 protein does not protect the *ex vivo* heart against infarction and (2) empagliflozin remains cardioprotective in an *in vivo* cardiac infarction model.
Methods: SGLT2 knockout mice on a C57Bl/6N background were created employing Crispr-Cas9 techniques. Isolated Langendorff-perfused mouse hearts of SGLT2 KO and their wild-type littermates were perfused with glucose, glutamine and palmitate, and subjected to 30 min ischaemia (I) and 90 min reperfusion (R). For the in vivo experiments, SGLT2 KO and their littermates were 7 days pretreated with empagliflozin (10mg/kg/d) or vehicle (5% DMSO), and on day 8 were subjected to 30 min in vivo LAD occlusion and 2 h reperfusion. In both the *ex vivo* and *in vivo* models the primary outcome was infarct size determined by the TTC technique. Data are presented as mean ± SE.
Results: Successful SGLT2 ablation was confirmed at the DNA, mRNA and protein levels in kidney. At 10 wks of age, the SGLT2 KO was functionally confirmed by high glucose in the urine, whereas plasma levels of glucose and ketones in the non-fasted state were unaffected. At baseline perfusion conditions in the Langendorff model, the SGLT2 KO was without consequences on cardiac performance: coronary flow, diastolic and systolic function, and rate-pressure product (heart rate x developed left ventricular pressure) were similar between genotypes. Following I/R, the recovery of cardiac performance was also similar between genotypes. The chronic deletion of SGLT2 did not result in the protection of hearts against infarction in the ex vivo heart model: infarct size amounted to 44 ± 5% and 41 ± 8% for WT hearts ($n = 19$) and SGLT2 KO hearts ($n = 17$), respectively. For the in vivo I/R models, infarct size in the vehicle-treated groups was 31.4 ± 1.2% for WT ($n = 8$), with comparable values observed for SGLT2 KO animals (31.0 ± 0.6%; $n = 4$). Pretreatment with empagliflozin resulted in similar significant protection against myocardial infarction for both WT and SGLT2 KO animals: infarct size in WT amounted to 22.9 ± 2.0% ($n = 7$) and to 23.1 ± 1.8% in SGLT2 KO mice ($n = 8$).
Conclusions: Chronic deletion of SGLT2 is not cardioprotective and Empagliflozin's cardioprotective effect against acute myocardial infarction is not through SGLT2 inhibition.

SYMPOSIUM 3 – DIABETES AND METABOLIC SYNDROME

57ASM – 014 | Sexual dimorphism in the modulation of energy balance in adipose tissue induced by exposure to obesogenic environments

D. Sousa[1,2,3]; A. Amaro[1,2,3]; M. Júnior Ferreira[4]; S. Pereira[2,5]; M. Rocha[1,2,3]; C. Barra[6]; R. Mello-Gomes[4]; P. Oliveira[2,5]; P. Matafome[1,2,3,7]
[1]Institute of Physiology and Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; [3]Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; [4]Department of Physiological Sciences, Institute of Biological Sciences, University Federal of Goiás, Goiâna, Brazil; [5]Centre for Neuroscience and Cell Biology, Coimbra, Portugal; [6]Hospitals of the University of Coimbra, Coimbra, Portugal; [7]Polytechnic Institute of Coimbra, School of Health Technology of Coimbra (ESTeSC), Coimbra, Portugal

Introduction: Early life obesity has been a major concern worldwide. Maternal obesity and postnatal overfeeding induce postnatal overweight, modulating energy balance mechanisms in liver and adipose tissue. Dysregulation of energy balance leads to an imbalance in energy storage and expenditure in visceral adipose tissue (VAT),

contributing to the development of metabolic syndrome in adulthood.

Objectives: Evaluate the sexual dimorphism in the modulation of peripheral energy balance in overfed female animals and females exposed to maternal obesity.

Methods: Two animal models were studied: (1) Offspring of dams submitted to a hypercaloric diet during pregnancy and lactation. (2) Postnatal overfed rats induced by litter reduction on the 3rd day after birth. In addition to lipid and glycaemic profiles, insulin receptor levels and NPY, ghrelin, and dopamine pathways were analysed in VAT and liver.

Results: Maternal obesity induces postnatal overweight in both sexes. In female VAT, NPY receptor-1 (NPY1R) is reduced, impairing lipogenesis which is counterbalanced by ghrelin lipogenic effects. In male both lipogenic and lipolytic processes were increased. In the liver, female offspring submitted to maternal obesity presented lower lipid oxidation-associated mechanisms [NPY1R, dopamine-1 receptor (D1R) and PPARα]. On the other hand, postnatal overfeeding induced by litter reduction did not induce postnatal overweight in females, corroborating their reduced VAT NPY/PPARγ signalling. In overfed males, adipogenesis was increased (NPY2R) while p-AMPK levels were reduced. In the liver, no molecular changes were observed; however, fat accumulation was noticed in the liver of female overfed rats.

Conclusion: The effects of postnatal overweight differ between male and female offspring. Male appears to be more susceptible to weight gain, while female present lipogenic/adipogenic processes reduction in VAT. The sexual dysmorphism may be related to distinct stimulation of different fat pads, since females are more prone to accumulate subcutaneous AT (SAT) while males store lipids predominantly in VAT.

57ASM – 015 | Neurochemical imaging identifies neuroprotection mechanisms counteracting excitotoxicity and neurovascular changes in the visual cortex of type 2 diabetic animal models

B. Caramelo[1,2,3]; T. Monteiro-Alfredo[1,2,3]; J. Martins[1,2,3,4]; J. Sereno[4,5]; J. Castelhano[4,6]; B. Manadas[2,6]; M. Castelo-Branco[4]; P. Matafome[1,2,3,7]

[1]Institute of Clinical and Biomedical Research (iCBR) and Institute of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]Centre for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; [3]Clinical Academic Center, Coimbra, Portugal; [4]Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), Institute for Nuclear Sciences Applied to Health (ICNAS), University of Coimbra, Coimbra, Portugal; [5]Coimbra Chemistry Centre (CQC), Chemistry Department, University of Coimbra, Coimbra, Portugal; [6]Centre for Neurosciences and Cell Biology (CNC), Faculty of Medicine, Polo 1, University of Coimbra, Coimbra, Portugal; [7]Instituto Politécnico de Coimbra, Coimbra Health School (ESTeSC), Coimbra, Portugal

Introduction: Obesity and type 2 diabetes (T2D) have metabolic and functional alterations in several brain regions. Nevertheless, their temporal evolution is still ambiguous. Hence, we intended to identify neurochemical mechanisms involved in the functional alterations in the visual cortex on obesity and T2D and their potential as clinical biomarkers.

Methods: Standard diet (SD) and high-fat diet (HFD)-fed Wistar rats (10 weeks, $n = 8$/group) constituted the control group (1-SD) and the obesity group (2-HFD), respectively. Simultaneously, rats from the T2D model (3-HFD + STZ) were fed with HFD (10 weeks) and injected with a low dose of streptozotocin (35 mg/kg, i.p., 4th week). Upon the treatment, the rats underwent proton magnetic resonance spectroscopy; and functional magnetic resonance imaging of primary visual cortex (V1), superior colliculus (SC) and lateral geniculate nucleus (LGN). The visual cortex was then collected for biochemical experiments. Statistical analysis was performed through one-way analysis of variance or Kruskal-Wallis test, depending on a normal or non-normal distribution of the samples, respectively; while correlations were evaluated by Pearson analysis.

Results: In contrast to SC and LGN, V1 presented a higher hemodynamic response in diabetic rats ($p = 0.226$ vs. SD), which was related with glutamate levels in the visual cortex ($r = 0.4491$, $p = 0.0316$). Despite the unaltered glutamate levels, glutamate-synthetising enzyme, glutaminase C, levels were enhanced in the HFD + STZ group ($p = 0.0613$ vs. SD). In this group, differently to gamma-aminobutyric

acid, taurine presented higher levels ($p = 0.00326$ vs. HFD) and was related ($r = 0.4459$, $p = 0.0380$) with the elevated glutamine ($p = 0.0252$ vs. SD). Taurine-agonistic GABA type A receptor (GABAAR) was also elevated ($p = 0.0211$ vs. SD, $p = 0.0153$ vs. HFD).

Conclusion: In T2D, V1 hyperactivity and consequent excitotoxicity seems to promote an adaptation mechanism through taurine and GABAAR in the visual cortex, prior to comorbidities like glycation, oxidative stress and cerebral atrophy.

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57ASM – 031 | Targeting senescence as a promising approach to treatment of type 2 diabetes mellitus and its comorbidities

P. Svoboda[1,2]; T. Havrlantova[1]; E. Vacurova[3]; J. Trnovska[1]; V. Skop[4]; K. Bohacova[3]; J. Stursa[3]; L. Werner[3]; J. Neuzil[3,5]; S. Stemberkova Hubackova[1]; M. Haluzik[6]

[1]Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Czech Republic; [3]Institute of Biotechnology, Czech Academy of Sciences, Prague, Czech Republic; [4]Diabetes, Endocrinology, and Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, USA; [5]School of Medical Science, Griffith University, Southport, Australia; [6]Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Introduction: Despite extensive research, limited efficacy of current therapies used for patients with obesity and type 2 diabetes mellitus (T2DM) have prompted a search for novel therapeutic options, such as targeting cellular senescence. Our goal was testing the MitoTam, a potential anti-cancer agent with senolytic activity, on pathologies related to T2DM.

Methods: C57BL/6 mice fed with standard or high fat diet for 8 month were treated with MitoTam (2 mg/kg body weight) given i.p. twice per week/4 weeks.

Results: Compared to non-treated mice MitoTam improved glucose parameters (fasting glucose 6.2 ± 1.1 mmoL/L vs. 9.8 ± 1.2 mmoL/L) and reduced body weight (35.6 ± 3.2 g vs. 45.2 ± 4.1 g), with most pronounced reduction of visceral adipose tissue (2.1 ± 0.4 g vs. 3.4 ± 0.3 g). Glucose-lowering effect of MitoTam was linked to improvement of T2DM-related hormones profile (insulin 0.4 ± 0.2 ng/mL vs. 2.3 ± 1.8 ng/mL; leptin 4.8 ± 1.9 ng/mL vs. 9.7 ± 1.1 ng/mL; GIP 0.7 ± 0.2 ng/mL vs. 1.2 ± 0.6 ng/mL) and was accompanied by reduced lipid accumulation in liver. Lower senescent cell burden in various tissues also resulted in lower level of circulating inflammatory mediators that enhance metabolic dysfunction. Moreover, decreased presence of senescent cells reduced kidney fibrosis, a frequently occurring complication of T2DM that worsen its progression. Mice treated with MitoTam showed lower accumulation of fibrotic cells and microenvironment in kidney followed by decreased kidney injury and secretion of injury molecules (KIM-1 98.8 ± 6.8 ng/mL vs. 82.1 ± 8.9 ng/mL; Cystatin C 1.71 ± 0.17 μg/mL vs. 1.54 ± 0.13 μg/mL) playing negative role in development of cardiovascular diseases in patients with T2DM.

Conclusion: Targeting senescence with MitoTam represents a novel approach to the treatment of T2DM and its related comorbidities. Since MitoTam has recently successfully carried out Phase 1/1b clinical trial for metastatic solid tumour patients with excellent safety profile, this compound has a potential to be translated into the clinic also for T2DM.

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57ASM – 033 | Prevalence of type 2 diabetes (T2D) in Lebanon: Association with inflammatory and infectious clinical markers

P. Chedid[1]; E. Sokhn[1,2]

[1]Beirut Arab University BAU, Beirut, Lebanon; [2]Lebanese Hospital Geitaoui University Medical Center, Beirut, Lebanon

Introduction and Background: This study aimed to determine the prevalence of type 2 diabetes (T2D) in Lebanon as well as its association with clinical markers of inflammation and infection.

Material and Methods: We examined in a cross-sectional manner the medical laboratory record of 4093 patients of all age groups and from all Lebanese regions between January 2021 and December 2021. The population was then divided into three groups based on HbA1C levels. Prevalence of T2D and its association with age, gender, calcium, vitamin D (VitD), neutrophils-to-lymphocytes ratio (NLR) and C-reactive protein (CRP) were determined. The prevalence of infection and its aetiology in a subpopulation of 712 patients was also assessed.

Results: The mean HbA1c was $5.9\% \pm 1.2$ and the prevalence of T2D defined as HbA1c > 6.5% was 16.8%. Risk factors independently associated with diabetes were age

(OR = 4.3; 95% CI = 2.32–8.09), gender (OR = 0.7; 95% CI = 0.55–0.98), triglycerides (OR = 2.5; 95% CI = 1.89–3.33), NLR (OR = 1.3; 95% CI = 1.01–1.79) and calcemia (OR = 2.0; 95% CI = 1.35–1.08). Prevalence of infections in a subgroup of 712 patients was 11.1%. E. coli urinary infection was the most common cause of infection (65.8%) with the highest prevalence in the pre-diabetic group (44.2%). Serum CRP level was higher in the diabetic group (22.5 mg/L ± 22.1) as compared to the pre-diabetic (7.9 mg/L ± 44.8) and control group (4.1 mg/L ± 9.3). Diabetic patients also presented the highest percentage of NLR > 3 (28.6%) as compared to pre-diabetic group (16.10%) and control group (14.4%).

Conclusions and Recommendations: The prevalence of T2D is increasing in the Lebanese population as compared to prior reports. These results should be taken into consideration to guide effective public health strategies aiming at preventing the rise of T2D in Lebanon. Identifying novel predictors of complications such as elevated NLR and CRP will also help improve the diagnosis and management of T2D and its complications.

57ASM – 057 | High-fat diet induces increased abundance of obesity-related genes in seminal vesicles of transgenerational mice model

B. Rodrigues[1,2]; S. C. Pereira[2,3]; M. G. Alves[2,3,4]; P. F. Oliveira[5]; R. L. Bernardino[2,3]

[1]Department of Medical Sciences, University of Aveiro, Aveiro, Portugal; [2]Clinical and Experimental Endocrinology, Unit for Multidisciplinary Research in Biomedicine, School of Medicine and Biomedical Sciences from University of Porto, Porto, Portugal; [3]Laboratory for Integrative and Translational Research in Population Health, Department University of Porto, Porto, Portugal; [4]Laboratory of Physiology, Department of Imuno-Physiology and Pharmacology, ICBAS – School of Medicine and Biomedical Sciences, University of Porto, Portugal; [5]LAQV-REQUIMTE and Department of Chemistry, University of Aveiro, Portugal

Introduction: Incidence of obesity is steadily rising worldwide. Evidence suggests a combination of obesity-inducing lifestyle with genetic factors may induce parental epigenetic alterations, passed on to descendants, increasing their likelihood for obesity and related pathologies.

Aim of the study: We aimed to evaluate the effects of a high-fat diet (HFD) on the expression of obesity-related genes (ORG) in seminal vesicles over generations.

Materials and Methods: We used a transgenerational mice model based on three generations (F0, F1, F2). Generation F0 was divided into three groups ($n = 6$ per group): control (standard chow – 200 days), HFD (200 days), and HFDt (HFD – 60 days, standard chow – 140 days). Generations F1 and F2 (sons and grandsons of F0) were fed standard chow for 200 days. The seminal vesicles of each animal were weighed, and RNA extracted to determine the abundance of transcripts of ORGs (MC4R, FTO, GNPDA2, TMEM18) through quantitative Reverse Transcriptase Polymerase Chain Reaction. The relative NA abundance of each group was normalized to housekeeping controls and expressed as fold variation to the control group of each generation.

Results: The expression of MC4R and GNPDA2 in the seminal vesicles of animals from the HFD and HFDt groups was significantly higher in the F0 generation compared to the control group, an effect not observed in subsequent generations. Meanwhile, TMEM18 expression exhibited a transgenerational increase in seminal vesicles of HFDt animals over three generations. The abundance of FTO transcripts in seminal vesicles did not appear affected by diet.

Conclusions: The study indicates that diets administered to F0 animals can affect the expression of ORGs in the next two generations. However, this detrimental impact was partially reversed in the F2 generation for MC4R and GNPDA2, critical players in metabolism. Therefore, a healthier diet may have long-term beneficial effects on the expression of ORG.

57ASM – 069 | Physiological variability in mitochondrial rRNA may predispose to metabolic syndrome

A. Pecinova[1]; P. Pecina[1]; K. Čunátová[1]; V. Kaplanová[1]; Msc. Guillermo Puertas[1]; Jan Šilhavý[2]; Marek Vrbacký[1]; Kateřina Tauchmannová[1]; Tomáš Čajka[3]; Michal Pravenec[2]; Josef Houštěk[1]; T. Mracek[1]

[1]Laboratory of Bioenergetics, Institute of Physiology, The Czech Academy of Sciences, Prague, Czech Republic; [2]Laboratory of Genetics of Model Diseases, Institute of Physiology, The Czech Academy of Sciences, Prague, Czech Republic; [3]Laboratory of Translational Metabolism, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Introduction: Obesity and its associated comorbidities, particularly metabolic syndrome (MS), are a growing concern in developed societies. Due to its polygenic nature, the genetic component of MS is only slowly being elucidated. Common mitochondrial DNA (mtDNA) sequence variants have been associated with late-onset human

diseases, including cardiovascular disease or type 2 diabetes mellitus, and may therefore be relevant players in the genetics of metabolic syndrome.

Results: In the current project, we investigated the effect of physiological variation in the mitochondrial DNA sequence on symptoms of metabolic syndrome using our unique models of conplastic rats carrying mtDNA from spontaneously hypertensive rat strain (mtSHR), Brown Norway strain (mtBN) or Fischer strain (mtF344) on the identical nuclear background (SHR). mtDNA sequence differences between these strains span structural genes for oxidative phosphorylation proteins, tRNAs as well as rRNAs. We found that mtBN, but especially mtF344, develop insulin resistance on a high-fat diet. This could not be explained by the changes in inflammatory markers or mitochondrial ROS production. To the contrary, it was associated with reduced oxidative capacity of heart, but not liver mitochondria. Reduced fatty acid oxidation led to the accumulation of bioactive diacylglycerols and subsequent inhibition of insulin signalling. We propose that in the case of mtF344 strain, these metabolic perturbations result from variation in the 12 s rRNA sequence, which affects mitoribosome assembly and subsequently mitochondrial protein translation.

Conclusion: Our work has demonstrated that physiological sequence variation in mitochondrial rRNA may be a relevant underlying factor in the progression of metabolic syndrome.

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57ASM – 074 | RF-amide peptide receptors GPR10 and NPFFR2 involvement in energy homeostasis and diet-induced obesity

N. N. R. Shekhar[1,2]; K. Bardova[1]; L. Maletinska[3]
[1]Laboratory of Adipose Tissue Biology, Institute Of Physiology CAS, Prague, Czech Republic; [2]Department of Animal Physiology, Faculty of Science, Charles University, Prague, Czech Republic; [3]Institute of Organic Chemistry and Biochemistry CAS, Czech Republic

Energy homeostasis as a balance between energy intake and expenditure is controlled by brain centers, mainly hypothalamus. RF-amide peptides control numerous biological functions such as regulation of food intake. The aim of the study is to characterize an effect of deletion of two of their receptors, GPR10 and NPFFR2, on energy homeostasis. We are using double knockout (GPR10 × NPFFR2; dKO) mice on a hybrid B6J × B6N background and their non-littermate controls, both males and females. Mice are fed either standard (STD; Ssniff RMH) or high-fat diet (HFD; based on lard) for 16 weeks starting at 4 months of age. Dual-energy absorptiometry is used to quantify bone mineral content, fat and lean body mass, and indirect calorimetry is used to quantify energy expenditure, energy intake, substrate partitioning, and physical activity at the beginning and end of this study. The goal is to detect possible differences which might precede or be a consequence of increased diet-induced obesity in dKO as compared to control mice. In the subsequent experiment, we will characterize the impact of an analog of a natural ligand of both receptors on body weight reduction. This experiment on mice after 16 weeks of high-fat-diet feeding will include 2-week intervention with application of either analog or control solution and will be performed along with a continuous measurement on indirect calorimetry. Both experiments are currently in progress with a planned end at the end of December. Consequently, data analysis together with the analysis of collected samples will be performed.

57ASM – 076 | Effect of a new laparoscopic partial jejuno-ileal diversion on bile acid levels and progression of type 2 diabetes mellitus

K. Reznickova[1]; P. Svoboda[2,3]; I. Modos[4]; S. Stemberkova Hubackova[2]; M. Vecka[5]; B. J. Kasperova[1]; I. Pleyerova[2]; T. Havrlantova[2]; R. Pavlovicova[2]; M. Cechova[2]; Z. Lacinova[2]; J. Klouckova[1]; M. Az[1]; J. Machackova[6]; P. Holeczy[7]; M. Buzga[8,9]; M. Haluzik[1,5]
[1]Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [3]Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Prague, Czech Republic; [4]Information Technology Division, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [5]Institute of Medical Biochemistry and Laboratory Diagnostics of the General University Hospital and of The First Faculty of Medicine of Charles University, Prague, Czech Republic; [6]Department of Internal Medicine and Cardiology, Division of Gastroenterology, University Hospital Ostrava, Ostrava, Czech Republic; [7]Department of Surgery, Vitkovice Hospital, Ostrava, Czech Republic; [8]Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic; [9]Institute of Laboratory Medicine, University Hospital Ostrava, Ostrava, Czech Republic

Obesity and type 2 diabetes mellitus (T2DM) are one of the most prevalent chronic diseases. New types of bariatric interventions, such as partial jejuno-ileal diversion

(PJID), are becoming popular for reduction of obesity. Bile acids play an important role in lipid absorption, however can serve as signalling molecules that modulate glucose metabolism. Our study was designed to investigate the effect of PJID on the alteration of bile acid levels in patients with obesity and T2DM.

Surgery was performed in 15 patients (5 males/10 females; age 47.6 ± 7.9 years; weight 117 ± 21 kg; BMI 42.1 ± 5.5 kg/m^2; 9 non-T2DM/6 T2DM) who were followed up for 24 months. Meal test was used to determine postprandial bile acid levels, which were detected by liquid chromatography with mass spectrometry, and assessment was based on area under the curve.

After PJID, weight loss was observed in all patients, and decrease in fasting glycaemia and glycated haemoglobin was also observed in patients with T2DM. Increase in serum levels of free bile acids and decrease levels of tau- and glycol-conjugated bile acids was observed in all patients, with the main change observed for chenodeoxycholic acid. In non-T2DM patients, there was increase in the levels of primary and free bile acids, with the main change observed for cholic acid. Reduced levels of glyco- and tau-conjugated ursodeoxycholic acid were found in T2DM patients. In all cases, this effect remained even 24 months after the surgery.

PJID appears to be effective in improving glycemic control and in weight reduction. The results of the study also suggest an effect of PJID on the spectrum of bile acids, which may contribute to the overall improvement of metabolic parameters of patients. Thus, bile acids may represent a potential target in the treatment of T2DM.

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57ASM – 081 | Obesity-associated adipose tissue (dys)function: Identifying new molecular markers

D. Rosendo-Silva[1,2,3]; B. Almeida[4]; P. Bastos Gomes[5]; S. Viana[1,2,3,6]; F. Reis[1,2,3]; R. Seiça[1,3]; P. Matafome[1,2,3,6]

[1]Institute for Clinical and Biomedical Research, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]Center for Innovation in Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; [3]Clinical Academic Center of Coimbra, Coimbra, Portugal; [4]Associação Protetora dos Diabéticos de Portugal, Lisboa, Portugal; [5]Surgery Service, Coimbra University Hospital, Coimbra, Portugal; [6]Polytechnic University of Coimbra, Coimbra Health School, Coimbra, Portugal

Introduction: The metabolic sequelae of obesity (metabolic syndrome and type 2 diabetes) are major public health challenges. Adipose tissue dysfunction is a hallmark of metabolic diseases and is characterized by the hypertrophic growth of adipocytes and insulin resistance, impairment of the angiogenic process, immune cells infiltration, and redox imbalance, although the mechanisms affected during the different stages of the metabolic dysregulation process are unknown.

Objectives: Identification of molecular markers of visceral adipose tissue dysfunction along the progression of the metabolic sequelae of obesity.

Methods: Patients with obesity ($n = 92$) at distinct stages of metabolic dysregulation, i.e., insulin sensitivity, insulin resistance, pre-diabetes, and type 2 diabetes, were recruited from the Obesity Consultation at the Coimbra University Hospital. Visceral adipose tissue samples were collected and the expression of several genes was evaluated by qPCR in the Biomark HD Fluidigm system. Data analysis was carried out using R software and GraphPad v.8.

Results: In the visceral adipose tissue, four relevant clusters of gene expression patterns were identified. Genes decreasing right after insulin resistance establishment were involved in angiogenesis and tissue remodelling, while pre-diabetes was associated with an increase in genes regulating the response to hypoxia and growth factors signalling. Type 2 diabetes was characterized by an increase in the inflammatory response, with concomitant downregulation of genes involved in hypoxia, insulin and redox response, and lipid metabolism.

Conclusions: Impairment in visceral adipose tissue plasticity is an early hallmark of tissue dysfunction in obesity, which is followed by a compensatory response to hypoxia in pre-diabetes. Once this effect is lost, adipose tissue dysfunction reaches a climax in type 2 diabetes, characterized by inflammation, inappropriate response to hypoxia, insulin, glutathione-dependent redox signalling, and lipid metabolism. Molecules/pathways suffering early dysregulation may become interesting therapeutic targets for treating obesity-associated metabolic complications.

57ASM – 082 | Peripheral crosstalk between Dopamine, Neuropeptide Y and Melanocortin systems

E. Lopes[1,2,3]; D. Rosendo-Silva[1,2,3]; P. Matafome[1,2,3,4]
[1]*Institute of Physiology and Coimbra Institute for Clinical And Biomedical Research (ICBR), Coimbra, Portugal;* [2]*Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal;* [3]*Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal;* [4]*Polytechnic University of Coimbra, School of Health Technology of Coimbra (ESTeSC), Coimbra, Portugal*

Introduction: Energy balance regulation depends on the communication between neuropeptide Y (NPY), melanocortin and dopamine systems at the central level. However, little is known about their peripheral actions towards regulation of energy balance, possibly being important for the development of new therapeutic strategies for obesity.

Objectives: Evaluate the interactions between NPY, Melanocortin and Dopamine systems at a peripheral level and their alterations in obesity.

Methods: Non-obese type 2 diabetic Goto-Kakizaki (GK) were fed with a high caloric diet (HCD) and treated with bromocriptine, an agonist for Dopamine Receptor 2 (D2R). Melanocortin and NPY receptors were determined in peripheral tissues. Samples of visceral adipose tissue (VAT) were collected from patients with obesity and with or without insulin resistance and diabetes. Gene expression of the different receptors was accessed by qPCR.

Results: The expression of NPY 1 Receptor (NPY1R) increased in patients with pre-diabetes ($p = 0.0460$) and decreased upon diabetes establishment ($p = 0.0242$). The expression of Dopamine 1 Receptor (D1R), NPY receptors 2 (NPY2R) and 4 (NPY4R), and Melanocortin Receptors 2 (MC2R), 4 (MC4R) and 5 (MC5R) decreases upon the establishment of insulin resistance. or prediabetes. D1R expression (Gs-coupled receptor) is positively related with other Gs-coupled receptors like NPY2R ($r = 0.692$), NPY4R ($r = 0.81$), and MC2R ($r = 0.79$), MC4R ($r = 0.806$) and MC5R ($r = 0.730$). Animals treated with bromocriptine showed an upregulation of NPY1R and MC4R levels in white adipose tissue (WAT), associated with both lipogenesis and catabolic activity, respectively.

Conclusion: The expression and correlation of these systems are altered in VAT upon insulin resistance establishment, being related with the metabolic sequelae of obesity. Pharmacological modulation of the Dopaminergic system apparently regulates both NPYergic and Melanocortinergic system in WAT, improving WAT lipid metabolism and suggesting possible bromocriptine's actions in obesity treatment.

57ASM – 100 | Significance of muscle non-shivering thermogenesis in obesity-resistant mouse model

S. Stanic[1]; P. Janovska[1]; P. Zouhar[1]; K. Bardova[1]; J. Otahal[1]; M. Vrbacky[1]; T. Acek[1]; K. Adamcova[1]; T. Cajka[1]; Z. Drahota[1]; A C. Rustan[2]; O. Horáková;[1]; J. Houstek[1]; M. Rossmeisl[1]; J. Kopecky[1]
[1]*Institute Of Physiology, Czech Academy Of Science, Praha, Czech Republic;* [2]*University of Oslo, Oslo, Norway*

Introduction: Nonshivering thermogenesis (NST) can significantly contribute to whole body energy expenditure and counteract development of obesity. Besides classical NST dependent on uncoupling protein 1 (UCP1) in brown adipose tissue (BAT), alternative putative NST mechanisms raise attention recently, namely those located in skeletal muscle. However, how muscle NST is regulated and whether it can be adaptive, remains a matter of long-lasting controversy.

Materials and Methods: Studying obesity-resistant A/J and obesity-prone C57BL/6J mice either adapted to thermoneutrality or acclimated to cold, we aim to uncover the role of UCP1-independent NST in model animals and the role of heart function in its regulation.

Results: Warm-acclimated C57BL/6J mice exhibited lower cold endurance than A/J mice, which was rescued by cold acclimation. However, adrenergic NST in BAT was induced by cold only in C57BL/6J mice and not in A/J mice. Neither shivering, nor physical activity could explain sufficient cold endurance in A/J mice. Lipidomic, proteomic, and gene expression analyses along with muscle palmitoyl carnitine levels and cytochrome c oxidase activity revealed cold-induced lipid oxidation exclusively in A/J mice. This could be explained by skeletal muscle NST, mediated by sarcolipin-induced uncoupling of activity of sarco(endo)plasmic reticulum calcium ATPase pump. Muscle NST may be regulated by changes in blood perfusion and dependent on heart function, as documented by our most recent set of results.

Conclusion: Impaired adrenergic NST in BAT of A/J mice could be compensated by NST in skeletal muscle. Muscle NST seems to be inducible by cold and it may provide not only protection from cold, but also obesity resistance, more effectively than BAT. Heart may play an important strain-specific role in regulation of muscular NST.

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57ASM – 110 | Therapeutic Potential of Umbilical Cord Tissue-derived Mesenchymal Stromal Cells in Diet-Induced Obesity and NAFLD

M. J. Meneses[1,2]; I. Sousa-Lima[1]; I. Ferreira[1]; H. Cruz[3]; J. M. Santos[3]; M. P. Macedo[1,4]
[1]iNOVA4Health, NOVA Medical School, NOVA University of Lisbon, Lisbon, Portugal; [2]DECSIS II Iberia, Lda, Évora, Portugal; [3]ECBio, Investigação e Desenvolvimento em Biotecnologia, S.A., Amadora, Portugal; [4]APDP – Diabetes Portugal, Education and Research Center, Lisbon, Portugal

Introduction: Mesenchymal Stromal Cells (MSCs) have shown promising therapeutic potential due to their immunomodulatory, anti-inflammatory, and regenerative properties. With a proprietary technique, a particular population of MSCs from umbilical cord tissue (UC-MSCs) was identified and has proven to be safe and effective in preclinical tests for several immune- and cardiovascular-related conditions. However, it is yet unclear how these UC-MSCs contribute to diet-induced obesity and non-alcoholic fatty liver disease (NAFLD). We hypothesize that UC-MSCs positively affect both glucose and lipid homeostasis, alleviating NAFLD.

Materials and Methods: Six weeks old male C57Bl/6J were divided into three groups to examine the effect of UC-MSCs: a control group given a normal diet, a group fed a hypercaloric diet (HFat), and a group given a hypercaloric diet treated with UC-MSCs (HFat-treated). The intervention was given weekly from week 11–18 by intraperitoneal injection of 10E6 cells. Insulin sensitivity and glucose tolerance were assessed after 5 weeks of dieting, and at the end of the study. The levels of insulin signalling pathway related proteins and lipid metabolism related genes were evaluated.

Results: HFat diet resulted in body weight gain, hyperglycemia, and glucose intolerance compared to the control group, indicating a prediabetic state. The HFat-treated group showed protection against body weight gain compared to the untreated HFat group, without affecting food intake. HFat-treated group presented lower random glucose levels and improved insulin sensitivity and glucose tolerance compared to HFat group ($p < 0.05$). The HFat-treated group presented decreased liver/body weight ratio and a reduction in cholesterol and triglyceride content in the liver compared to the HFat group.

Conclusion: Our data suggests that these UC-MSCs have a protective role in diet-induced obesity, prediabetes profile and NAFLD. However, further investigation is required to determine the mechanism through which UC-MSCs protect against insulin signalling and hepatic lipid accumulation.

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57ASM – 117 | Insulin and C-Peptide measurements variations: The influence of evaluation methods and brands and clinical implications

R. S. Patarrão[1]; M. J. Meneses[1,2]; A. Romão[2]; J. F. Raposo[3]; M. P. Macedo[1,3]
[1]iNOVA4Health, NOVA Medical School|Faculdade de Ciências Médicas, NMS|FCM, Universidade NOVA de Lisboa, Lisboa, Portugal; [2]DECSIS II Iberia, Évora, Portugal; [3]APDP – Diabetes Portugal, Education and Research Center, Lisboa, Portugal

Introduction: Diabetes diagnosis has been mainly focused on glycemia and glycated haemoglobin measurements. It has become evident that quantification of other biochemical parameters, such as insulin and C-peptide, offers a wider view of each individual's metabolic status, allowing a precision medicine approach, since diabetes management and therapy goes beyond glucose control. Our aim was to assess the reproducibility of results on insulin and C-peptide levels' when using different methods and/or the same method from different brands.

Material and Methods: Subjects recruited at APDP clinic underwent a 75 g OGTT and blood samples were collected at baseline, 30, 90, and 120 min after the OGTT. Insulin and C-peptide were measured by chemiluminescence and three commercially available ELISA kits from different brands (1, 2 and 3).

Results: Chemiluminescence measurement for both insulin and C-peptide resulted in higher levels than ELISAs ($p < 0.05$). Concerning insulin, there were differences in the results obtained both between the different ELISAs and the chemiluminescence assay. Although in the detection range, ELISA 3 did not perform well below 40μIU/ml. In contrast, ELISA 1 was very sensitive, and a dilution was needed.

There were also inconsistencies in C-peptide results, but less than with insulin. In general, ELISA 1 seemed to be the most consistent. ELISA 2 was the only one that shows peaks at 90 min, with all the other following the same trend between them, although with slightly different levels: chemiluminescence assay gives the highest values, followed by ELISA 1 and then by ELISA 3.

Conclusions: We observe that different methods and brands do not provide consistent results between them, and therefore, the evaluation of results may be biased. This should be taken into consideration when using these results for disease management evaluation or further used

in calculation of indexes of insulin secretion, resistance and/or metabolism.

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57ASM – 118 | Role of insulin and Uncoupling protein 1 in glucose lowering effect of β3-adrenergic stimulation

P. Zouhar

Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

Introduction: Stimulation of β3-adrenergic receptors directly augments glucose uptake into brown adipocytes through mechanisms distinct from those utilized by insulin. We have here examined to what extend this property of β3-adrenergic agonist can be utilized to lower plasma glucose levels in diabetic mice models with diminished capacity for insulin-stimulated glucose disposal.

Methods: We acutely applied β3-adrenergic agonist CL 316243 to several models of insulin resistance (db/db mice, dietary obese mice) and insulin deficiency (streptozotocin treatment), monitoring its effect on blood glucose and insulin levels.

Results: The β3-adrenergic agonist CL 316243 markedly lowered plasma glucose levels and increased 2-deoxyglucose uptake into brown adipose tissue in euinsulinemic mice, but it failed to lower blood glucose in db/db, dietary obese, and streptozotocin-treated mice, although its effects on energy expenditure were preserved. Changes of blood glucose did not differ between UCP1-deficient and control animals.

Conclusion: Despite the ability of CL 316243 to stimulate glucose uptake into brown adipocytes, the systemic glucose-lowering effect of CL 316243 is dependent on the presence of insulin but not of UCP1. Even in the absence of its glucose-burning properties, brown adipose tissue may be an important mediator of the glucose-lowering effects of β3-adrenergic stimulation.

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57ASM – 122 | Role of ISL1 in pancreatic endocrine cell development

V. Fabríciová; R. Bohuslavová; G. Pavlínková; L. Lebrón-Mora

Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic

Introduction: Functional pancreatic α and β cells are necessary for glucose homeostasis. The molecular mechanisms of the generation and maturation of these cells are still unclear. Our study is focused on the role of transcription factor Isl1 during pancreas development.

Material and Methods: We generated a transgenic mouse model of conditional knockout of the Isl1 gene using the Cre-loxP system (Neurod1-Cre, Isl1f/f) with fluorescent reporter tdTomato. RNA-seq, Cut&Tag-seq, and RT-qPCR were performed on FACS-sorted cells. We used immunohistochemistry and confocal microscopy to evaluate the cellular pancreatic endocrine phenotype. for a better insight into the anatomical 3D microenvironment, we performed light sheet fluorescent microscopy. Bioinformatic tools were used to identify ISL1 binding sites (HOMER) and to deconvolve cell subtypes based on single-cell transcriptomic dataset (CibersortX).

Results: Isl1CKO mice show diabetic phenotype with significant neonatal hyperglycemia that increased with age. Isl1CKO showed complete loss of α cell lineage and disrupted architecture of the islets of Langerhans. β cell mass was significantly reduced compared to controls. RNA-seq data show dysregulated transcriptome in Isl1CKO-downregulated genes important α and β cell markers and regulators, upregulation of endocrine progenitor specific genes. These data were confirmed by RT-qPCR. The Cut&Tag method that uncovers the epigenetic landscape revealed altered silencing by H3K27me3 histone modification in the promoter regions in genes essential for endocrine cell differentiation. HOMER analysis indicates that downregulated genes with H3K27me3 enrichment, such as Mafa and Eya1 (mature β cell markers), and upregulated genes without H3K27me3 modification, like Vim, Fev and Pbxip1 (endocrine progenitor cell marker), contain ISL1 binding motif.

Conclusions: Isl1 is a critical transcription and epigenetic modulator for the generation and maturation of fully functional α and β cells. More studies, especially single-cell transcriptomics, are needed to understand molecular pathways governed by ISL1.

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57ASM – 163 | Comparative efficacy of ticagrelor in patients with coronary artery disease and type 2 diabetes mellitus after percutaneous coronary interventions

D. Orziev; A. Abdullaev; B. Alyavi; J. Uzokov; O. Mukhitdinova
Republican Specialized Scientific Practical Medical Center of Therapy and Medical Rehabilitation, Tashkent, Uzbekistan

Introduction and Background: Despite the use of approved doses of the antiplatelets major adverse cardiac events (MACE) frequently occur in short and mid-term period in patients with type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD). Aim of the study was to evaluate the efficacy of ticagrelor in patients with CAD and T2DM after elective percutaneous coronary interventions (PCI).

Material and Methods: The prospective study with 112 patients with CAD and T2DM who admitted for the elective PCI were enrolled in the study from 2018 to 2022 (aged 48–73 years, mean age 58.6 ± 13.4 years, male 54%). Patients were divided into two groups by 56 via open randomization. Group I patients were assigned ticagrelor plus aspirin whilst Group II were assigned clopidogrel plus aspirin for 1 year. Platelet aggregation was assessed at baseline and the loading dose of antiplatelet and safely were assessed during the follow up.

Results: Inhibition of platelet aggregation (IPA) with 20 μmoLL ADP at 12 h was significantly higher in ticagrelor group than clopidogrel group (71.78 ± 14.31% vs. 42.85 ± 18.33%, $p < 0.001$). During the maintenance dose, IPA with 20 μmoLL ADP at 48 h was significantly higher in ticagrelor group than clopidogrel group (68.12 ± 13.27% vs. 45.82 ± 17.54%, $p < 0.001$). PCI bleeding complications were similar in each group and there was not observed any statistically significant changes ($p > 0.05$). During the mean follow-up period ticagrelor group tended to have higher bleeding than clopidogrel group, however they were not observed any statistically significant changes (log-rank test; 0.752). Pertaining to MACE, ticagrelol group tended to have lesser MACE than clopidogrel group, ($p = 0.045$). 56 ticagrelor treated patients showed that MACE tended to be negatively associated with post PCI bleeding complications ($p = 0.048$).

Conclusions and Recommendations: DAT with ticagrelor plus aspirin is superior than clopidogrel plus aspirin to prevent MACE in patients with CAD and T2DM after PCI.

57ASM – 166 | Thermoneutral housing potentiates NAFLD progression in C57BL/6N mice fed standard but not high-fat diet

M. Rossmeisl[1]; O. Horáková[1]; G. Sistilli[1]; K. Bardova[1]; M. Mitrovic[1]; T. Cajka[1]; K. Lackner[2]
[1]Institute of Physiology of the Czech Academy of Sciences, Prague 4, Czech Republic; [2]Institute of Pathology, Medical University of Graz, Graz, Austria

Introduction: Non-alcoholic fatty liver disease (NAFLD) is associated with obesity and can progress to more severe stages such as steatohepatitis and fibrosis. Thermoneutral housing in conjunction with high-fat feeding was found to promote the development of obesity-associated NAFLD and its progression to steatohepatitis in C57BL/6J mice. Interestingly, although C57BL/6N mice represent another frequently used substrain in metabolic research, the effect of thermoneutral environment on NAFLD progression in these animals is unknown.

Materials and Methods: Male C57BL/6N mice fed either a standard (STD) or high-fat diet (HFD; fat ~60 kJ %) for 24 weeks and housed in either a standard (22°C) or thermoneutral (30°C) environment were used. Standard histological and molecular analyses were used along with comprehensive metabolipidomic profiling of the liver.

Results: HFD administration promoted weight gain and hepatic steatosis, regardless of ambient temperature. Histological scores of hepatic inflammation and fibrosis were relatively low (<1.0) in HFD-fed mice in both temperature groups. Hepatic gene expression of inflammation and tissue remodelling markers was generally increased in HFD-fed mice, with occasional stimulatory effects of thermoneutral housing (Cd68, Ccl2, Timp1, Thbs1). Liver metabolomic profiling in HFD-fed mice revealed the involvement of autophagy-related metabolites in NAFLD development, but not the effect of ambient temperature. In STD-fed mice, thermoneutral housing increased weight gain, adiposity, hepatic steatosis, and NAFLD activity score. This was accompanied by increased de novo lipogenesis and changes in hepatic metabolome characterized by a complex decrease in phospholipid species and metabolites involved in the urea cycle and oxidative stress defence.

Conclusion: Thermoneutral conditions appear to promote NAFLD progression depending on the C57BL/6 mouse substrain and/or the amount of dietary fat. Our results may help in selecting a suitable strain for future experiments aimed at inducing progressive NAFLD.

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57ASM – 170 | Circular dorsal ruffles and insulin receptor trafficking: Pioglitazone as a restorative agent

A. Teshima[1]; M. Araujo-Correia[1]; L. M. Oliveira[1]; D. Barral[1]; R. Machado de Oliveira[1]; M. P. Macedo[1,2]
[1]iNOVA4Health, NOVA Medical School, Faculdade de Ciencias Medicas, Universidade NOVA de Lisboa, Lisbon, Portugal; [2]Department of Medical Sciences, University of Aveiro, Aveiro, Portugal

Background and Aims: Impaired insulin signalling is linked to insulin resistance, key factor in the pathology of diabetes. While insulin receptor (InsR) internalization is vital for proper response to insulin, not much is known about it. We observed that in hepatocytes, lnsR is internalized through actin-rich structures – circular dorsal ruffles (CDRs) which form upon insulin stimulation. Interestingly, chronic exposure to high insulin levels impairs CDRs formation.

We hypothesized that inhibition of CDRs formation by high insulin compromises InsR internalization, subsequent trafficking to early endosome (EE) and recycle to the cell membrane. Importantly, it is unknown whether the insulin sensitizer pioglitazone (PIO) modulates InsR endocytosis through CDRs.

Material and Methods: CDR formation was examined in Hepa 1–6 mouse hepatoma cells maintained in normal medium or in medium with 200 nM of insulin for 48 h. Cells were stimulated with 100 nM insulin for several timepoints. To observe early endosomes (EE) and recycling endosomes (RE), cells were labelled with EEA1 and Rab11a, respectively. 20 μM PIO was added to the hyperinsulinemia condition for 24 h and then stimulated with 100 nM insulin.

Results and Conclusion: In control condition, InsR colocalizes with EEA1 peaking at 5 min. While in hyperinsulin condition we observed a decreased colocalization in every timepoint, suggesting an impairment in InsR endocytosis, concomitant with CDR formation blockage and insulin pathway inactivation. Moreover, recycling to the plasma membrane in hyperinsulin condition is impaired. We show for the first time that hyperinsulinemia inhibits lnsR internalization through CDRs and subsequent trafficking to EE and RE. Our results suggest that abrogation of CDRs formation contributes to the onset and/or exacerbation of insulin resistance. Moreover, in the presence of PIO, CDRs formation recovered to similar levels of control. In the future it would be interesting to look at molecules involved in CDR formation as promising therapeutic targets for diabetes.

57ASM – 173 | Alterations in brain stiffness and metabolism in Western Diet-induced metabolic syndrome: Exploring the interplay between mechanotransduction and metabolic pathways

H. Gerardo[1,2]; R. Amorim[1,3]; I. C. M. Simões[4]; C. Aveleira[5]; C. Cavadas[1]; M. R. Wieckowski[4]; P. J. Oliveira[1]; M. Grãos[1]; J. Teixeira[1]
[1]CNC – Center for Neuroscience and Cell Biology, CIBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [2]Doctoral Programme in Pharmaceutical Sciences, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; [3]CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal; [4]Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland; [5]MIA – Multidisciplinary Institute of Aging, University of Coimbra, Coimbra, Portugal

Introduction: Metabolic syndrome (MetS) affects a quarter of the world's population and is a major public health concern. MetS is a clustering of several disorders including dyslipidemia, obesity and hyperglycemia/insulin resistance and every component has been linked to an increased risk of neurodegenerative diseases like Alzheimer's and dementia. Although mechanisms behind these diseases are not fully understood, impairment in tissue mechanics have been observed. This includes specifically a decrease in brain stiffness, which can affect metabolic pathways. Our study aims to investigate the role of mechanotransduction and metabolic dysfunction as initiators and/or potentiators of MetS to establish a potential cause-effect relationship between brain stiffness changes and metabolic alterations disease.

Materials and Methods: C57BL/6J mice were fed with standard diet (SD) or Western diet (WD) (30% high-fat, 30% high-sucrose) for 16 weeks. In vitro, mouse neuronal cell-line (HT22) was cultured on polyacrylamide hydrogels with physiological (~6.5 kPa) or dementia brain rigidity (~2.5 kPa). Levels of markers for brain stiffness, mitochondria, glucose, and fatty acid metabolism were assessed by immunoblot using brain/cell homogenates.

Results: We observed decreased levels of proteins related with brain stiffness in WD compared with SD-fed mice (α-SMA, cofilin 30% and CTGF 30%) but no alterations on mitochondrial markers (TOM20, VDAC, ATP5). Notwithstanding, we noticed increased levels of glucose metabolism markers (GLUT1 200%, HK 30%), and upregulation of mitochondrial beta-oxidation marker (CPT1 125%) in brains of WD-induced MetS. Interestingly, in

vitro, we observed no significant differences in mitochondrial or fatty acid-oxidation markers (TOM20, ATP5, CPT1), but there is an overall decay of protein levels associated with metabolism (Complex IV 25%, HADHA 55%, HK 80%) when cells were cultured on soft substrate (2.5 kPa) compared to physiological stiffness (6.5 kPa).

Conclusion: This study suggests that primary alterations in brain lipid metabolism may lead to subsequent changes in brain tissue mechanics in mice with WD-induced MetS.

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57ASM – 178 | Cervico-facial area skin AGEs assessment by means of High Frequency Ultrasound

A. M. Băbțan
Oral Rehabilitation Department, Iuliu Haţieganu University Of Medicine And Pharmacy, Cluj-Napoca, Romania

Aim. The aim of the study was to establish if there is a correlation between High Frequency Ultrasound (HFU) measurements of cervico-facial area skin and advanced glycation end products (AGEs).

Material and Methods: The present study included 102 subjects. Anthropometric parameters and disease history were recorded, and blood samples were harvested in order to assess biochemical parameters of Sun-exposed skin (zygomatic region) was assessed using HFU (DUB® cutis, Taberna Pro Medicum) with a 22 MHz probe.

Results. The epidermis depth was directly correlated with methylglyoxal ($r = 0.204$; $p = 0.04$). The depth of UV aged dermis was directly correlated with methylglyoxal ($r = 0.283$; $p = 0.003$), carboxymethyl-lysine ($r = 0.301$; $p = 0.001$) and fructosyl-lysine ($r = 0.191$; $p = 0.04$). The number of pixels was directly correlated with methylglyoxal ($r = 0.197$; $p = 0.03$), carboxymethyl-lysine ($r = 0.240$; $p = 0.01$). The density of UV aged dermis was indirectly correlated with fructosyl-lysine ($r = -0.268$; $p = 0.004$). The subcutaneous tissue depth was directly correlated with fructosyl-lysine ($r = 0.269$; $p = 0.004$). The subcutaneous number of pixels was directly correlated with carboxymethyl-lysine ($r = 0.205$; $p = 0.03$).

Conclusions: The process of skin aging is strongly related to the AGEs accumulation. HFU could represent an important noninvasive tissue aging evaluation and monitoring in cervico-facial area.

57ASM – 197 | WWOX inhibition by Zfra1-31: A new therapeutic approach against type 2 diabetes-associated brain damage

S. C. Correia[1,2,3,†]; S. Cardoso[1,2,3,†]; M. Laranjo[1,2,4]; P. Moreira[1,2,5]; C. Carvalho[1]
[1]*Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal;* [2]*Institute for Interdisciplinary Research (III), University of Coimbra, Coimbra, Portugal;* [3]*Center for Innovation in Biomedicine and Biotechnology (CIBB), Coimbra, Portugal;* [4]*PhD Program in Experimental Biology and Biomedicine (PDBEB), University of Coimbra, Coimbra, Portugal;* [5]*Institute of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal*
[†]*Equally contributed*

The world is facing the rise of a silent pandemic, type 2 diabetes (T2D). It is far known that T2D substantially increases the risk to develop neurodegenerative diseases. In this line, it becomes urgent to clarify the mechanisms underlying T2D-associated brain damage to find effective strategies to avoid/delay its development. We hypothesize that ww domain-containing oxireductase 1 (WWOX) is a potential therapeutic target in the diabetic brain and that Zfra1-31 peptide, a specific inhibitor of WWOX, has therapeutic potential against diabetes-induced neurodegeneration. Our study was performed in a high fat diet (HFD)-induced T2D mice treated or not with 2 mM Zfra1-31 for 4 weeks (4×; 1 injection/week via tail vein). Mice were subdivided and half were sacrificed 2 weeks after the last Zfra1-31 injection (short-term) and the remaining were sacrificed 2 months later (long-term). We performed a battery of behavioural and cognitive tests and assessed brain cortical mitochondria function and oxidative stress levels. HFD induced a phenotype of T2D characterized by an increase in body weight and peripheral blood glucose levels and inflammatory markers. T2D mice also showed increased levels of anxiety and cognitive defects. Diabetic brain cortical mitochondria presented defects in the respiratory chain and phosphorylative system, decreased calcium buffering capacity and increased oxidative stress levels. Interestingly, Zfra1-31 treatment decreased occasional blood glucose levels, anxiety-like behaviour, and memory impairment. Furthermore, Zfra1-31 improved mitochondrial respiratory chain and phosphorylation system function and calcium buffering capacity and decreased oxidative stress, being these defects associated to an inactivation of WWOX. Our observations demonstrate that HFD promotes brain cortical mitochondria dysfunction and oxidative stress contributing to behavioural and cognitive alterations. More, our findings

support the therapeutic potential of Zfra1-31 against diabetes-associated brain damage.
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57ASM – 214 | Endothelial functional status in patients with atherosclerotic coronary artery disease and type 2 diabetes mellitus after COVID-19

S. Ubaydullaev; A. Alyavi
Republican Specialized Scientific Practical Medical Center of Therapy and Medical Rehabilitation, Tashkent, Uzbekistan

Introduction: Aim of the study was to estimate endothelial functional status in patients with atherosclerotic coronary artery disease (ASCAD) and type 2 diabetes mellitus (T2DM) who underwent Covid-19.
Material and Methods: 65 patients with ASCAD and T2DM after coronavirus infection (Group I) and 65 patients with ASCAD and T2DM without any history of Covid-19 (Group II) were enrolled in this study. Group I patients aged 45–73 years, mean age 61.3 ± 11.8 years; male = 48% and Group II patients aged 41–76 years, mean age 62.5 ± 13.8 years; male = 46%. Endothelial functional status was assessed by flow-mediated vasodilation of brachial artery (FMD). All statistical analysis were performed by STATA software.
Results: FMD of brachial artery was significantly decreased in patients with ASCAD and T2DM after Covid-19 than those patients without Covid-19 ($p < 0.01$). There were a correlation between Covid-19 and reduced FMD in Group I ($r = 0.7$, CI 95%, $p = 0.032$). When we assessed systolic function of the left ventricle, there were a positive correlation between reduced FMD and low ejection fraction ($r = 0.6$, CI 95%, $p = 0.028$); however, this correlation were more pronounced in Group I ($p = 0.001$). Multivariate analysis revealed that reduced flow-mediated vasodilation of brachial artery was independent predictor of poor systolic function of patients with ASCAD and T2DM especially in those after Covid-19 (odds ratio [OR] 1.52, $p = 0.026$). When we separately analysed between men and women there were not any statistical significant changes between male and female ($p > 0.05$).
Conclusion: Patients with atherosclerotic coronary artery disease and T2DM after Covid-19 had impaired FMD of brachial artery. Even though, there were positive correlation between flow-mediated vasodilation of brachial artery and reduced ejection fraction, patients with ASCAD and T2DM after Covid-19 had strong correlation of it. In atherosclerotic coronary artery disease with type 2 diabetes mellitus who have underwent Covid-19, FMD independently associated with poor left ventricular systolic function.

57ASM – 216 | Influence of dapagliflozin on lipid parameters in patients with coronary artery disease and type 2 diabetes mellitus

N. Kayumov; B. Alyavi
Tashkent Paediatric Medical Institute, Tashkent, Uzbekistan

Introduction: Sodium glucose co-transporter 2 inhibitors (SGCT-2) have shown several favourable effects in patients with type 2 diabetes mellitus (T2DM). Aim of the study was to evaluate influence of sodium glucose co-transporter 2 inhibitor – dapagliflozin on lipid spectrum in patients with coronary artery disease (CAD) and T2DM.
Methods: 86 patients with CAD and T2DM were enrolled in the prospective study (aged 45–73 years, mean age 61.2 ± 12.54 years, male = 54%). Patients were divided into 2 groups and Group I were assigned dapagliflozin along with standard therapy and Group II only standard therapy. Anthropometry, laboratory and instrumental data were assessed at baseline and after the 12 weeks of the treatment. All statistical analysis were performed using STATA software.
Results: During the treatment total cholesterol has been decreased in each group from baseline; however, there were not statistically significant changes between groups ($p > 0.05$). High-density lipoprotein – cholesterol (HDL-C) has been improved in Group I than Group II (21% vs. 11%, $p < 0.05$). Low-density lipoprotein-cholesterol (LDL-C) and remnant cholesterol have been reduced significantly in Group I than group II ($p < 0.05$). Besides, non HDL-cholesterol has been decreased significantly in Group I than Group II ($p < 0.05$). Regarding the triglycerides there were not statistically significant changes between groups ($p > 0.05$). When we analysed by gender there were not observed any statistically significant changes between sexes ($p > 0.05$).
Conclusion: SGCT-2 – dapogliflozin improve HDL-C and reduce non-HDL and remnant cholesterol in patients with CAD and T2DM. Further studies are needed with large amount of patients to understand exact mechanism.

57ASM – 231 | Metabolic effects of *n*-3 fatty acids administered as Calanus oil in dietary obese mice with modified PPARα expression

O. Horakova; V. Kalendova; T. Cajka; M. Rossmeis
Laboratory of Adipose Tissue Biology, Institute of Physiology of Czech Academy of Science, Prague, Czech Republic

Introduction: The n-3 polyunsaturated fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA), act as endogenous ligands for the transcription factor PPARalpha, an important regulator of genes involved in hepatic lipid metabolism. We aimed to determine how metabolic effects of Calanus oil, a wax ester-bound EPA and DHA source, depend on the presence of functional PPARalpha.

Methods: Twelve-week-old male 129S1/SvImJ mice, wild-type (WT) and PPARalpha-deficient (KO) mice, were fed either a standard diet or a high-fat corn oil-based diet (cHF; 32%wt as lipids) for 8 weeks. To supplement EPA and DHA, 15% of the cHF diet lipids were replaced with Calanus oil (cHF+CO). We quantified tissue lipids and analysed gene expression and the metabolipidome in liver. Glucose production and its suppression by insulin were evaluated in isolated hepatocytes.

Results: Administration of cHF resulted in a threefold higher liver fat content in KO animals compared to WT (WT, 82±4 vs. KO, 256±13 mg/g tissue). As expected, expression of genes involved in beta-oxidation and levels of acylcarnitines and coenzyme Q were reduced in cHF-fed KO mice. Notably, feeding cHF+CO reduced hepatic steatosis in the KO group but did not affect liver fat content in WT mice. Regardless of genotype, cHF+CO-fed mice had an altered composition of various triacylglycerol and phospholipid species compared to their cHF-fed counterparts. Hepatocytes isolated from cHF-fed KO (but not WT) mice exhibited impaired glucagon-stimulated glucose production and its suppression by insulin, a phenotype that was not rescued in hepatocytes isolated from KO animals fed cHF+CO.

Conclusion: Chronic Calanus oil administration may alter hepatic lipid profile independently of functional PPARalpha presence. However, its beneficial effects may only occur upon conditions of significantly increased hepatic steatosis, probably due to some indirect mechanisms induced outside the liver.

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57ASM – 240 | NOX4 deletion ameliorates obesity-induced bone impairment and decreases bone marrow adiposity in animal model of obesity

M. Dzubanova[1,2,*]; J. M. Bond[3,*]; M. Ferencakova[1]; A. Benova[1,2]; A. K. Addington[3]; G. Kerckhofs[4,5,6]; G. Pyka[4]; T. Cajka[7]; M. Kassem[8]; C. M. Andreasen[9]; T. L. Andersen[9]; S. M. Craige[3]; M. Tencerova[1]
[1]Laboratory of Molecular Physiology of Bone, Institute of Physiology of the Czech Academy of Sciences, Prague 142 20, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Human Nutrition, Foods and Exercise; Virginia Tech, Blacksburg, VA 24061, USA; [4]Biomechanics lab, Institute of Mechanics, Materials, and Civil Engineering, UCLouvain, Louvain-la-Neuve, Belgium; [5]Institute for Experimental and Clinical Research, UCLouvain, Woluwe, Belgium; [6]Department of Material Science and Engineering, KU Leuven, Leuven, Belgium; [7]Laboratory of Translational Metabolomics, Institute of Physiology of the Czech Academy of Sciences, Prague 142 20, Czech Republic; [8]KMEB, University of Southern Denmark and Odense University Hospital, Odense, Denmark 5000; [9]Department of Pathology, University of Southern Denmark, Odense, DK 5000

*Equally contributed to this work.

Introduction: Obesity causes increased accumulation of adipose tissue not only in periphery but also in bone marrow (BMAT). BMAT expansion is accompanied with higher ROS production causing oxidative stress leading to increased risk of bone fractures and bone loss. NADPH oxidase 4 (NOX4) is a major ROS producer affecting differentiation potential of cells. Thus, we hypothesize that deletion of NOX4 may affect bone marrow mesenchymal stem cells (BM-MSCs) properties and bone homeostasis.

Methods: WT and Nox4-/- male mice were fed chow or 60% high-fat-diet (HFD) ($n = 5$–12 per group) for 5 months and examined for metabolic (glucose tolerance test, body composition) and bone parameters (mCT, BMAT evaluation). In addition, bioenergetic profile of primary BM-MSCs was performed.

Results: In HFD condition, Nox4-/- mice gained less body weight and had less fat mass compared to WT (45.3 g vs. 32.2 g, $p < 0.0001$; 28.1% vs. 16.8%, WT vs Nox4-/-; $p < 0.0001$) with no significant effect on glucose tolerance. Further, Nox4-/- mice showed increased cortical thickness and decreased porosity (~6%, $p < 0.05$, ~30% $p < 0.05$, respectively) in comparison to WT in chow and HFD. In addition, Nox4-/- mice manifested higher trabecular thickness in linear size with a reduced trabecular number (~20%, $p < 0.05$; ~13%, $p < 0.05$; respectively) accompanied with decreased BMAT volume in HFD Nox4-/- compared to WT mice. Interestingly, primary mBM-MSCs isolated

from chow Nox4-/- mice under an acute stress condition of high glucose (25 mM) exhibited lower basal and maximal oxygen consumption rates (50%, $p < 0.01$; 32%, $p < 0.05$, respectively) and increased glycolysis compared to WT mBM-MSCs. Moreover, downregulation of NOX4 in hBM-MSCs-TERT led to increased ALP activity and decreased adipogenesis (95%, $p < 0.01$; 45% $p < 0.01$) suggesting its role in regulation of metabolism and differentiation of hBM-MSCs.

Conclusion: Taken together, these data suggest an important role of NOX4 in regulation of bone-fat homeostasis in obesity.

Keywords: obesity-induced bone loss, bone marrow adipose tissue, bone marrow mesenchymal stem cells, adipogenesis, NADPH oxidase 4.

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SYMPOSIUM 4 – HEPATOLOGY AND GASTROENTEROLOGY

57ASM – 009 | Waist to height ratio in non-alcoholic fatty liver disease – A systematic review and meta-analysis

A. Ismaiel[1]; B. El Hosiny[2]; M. Ismaiel[3]; D.-C. Leucuta[4]; S.-L. Popa[1]; C. Sorina Catana[5]; D. L. Dumitrascu[1]

[1]2nd Department of Internal Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3]Cardiothoracic Surgery department, Royal Victoria Hospital, Belfast, United Kingdom; [4]Department of Medical Informatics and Biostatistics, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [5]Department of Medical Biochemistry, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Background and aims: The waist-to-height ratio (WHtR), a simple obesity indicator obtained by dividing waist circumference by height, is not addressed in the current non-alcoholic fatty liver disease (NAFLD) guidelines. Accordingly, we conducted a systematic review and meta-analysis aiming to assess WHtR in NAFLD.

Methods: We identified observational studies assessing WHtR in NAFLD by performing a systematic electronic search on PubMed, Embase, and Scopus. The quality of included studies was evaluated using the QUADAS-2 tool. The two main statistical outcomes were the mean difference (MD) and area under the curve (AUC).

Results: A total of 27 studies were included in our quantitative and qualitative synthesis, involving a total population of 93,536 subjects. Included studies were conducted in Europe, Asia, South America, and the Middle East. Compared to controls, the WHtR was significantly increased in NAFLD patients (MD 0.073 [95% CI 0.058–0.088]). These findings were maintained after conducting a subgroup analysis according to the diagnosis method of hepatic steatosis, using ultrasound (MD 0.066 [96% CI 0.051–0.081]) and transient elastography (MD 0.074 [96% CI 0.053–0.094]). Furthermore, NAFLD male patients had significantly decreased WHtR compared to female patients (MD -0.022 [95% CI -0.041 – −0.004]). The AUC for predicting NAFLD using WHtR was 0.815 (95% CI 0.780–0.849).

Conclusions: WHtR is significantly increased in NAFLD patients compared to controls. Compared to NAFLD male patients, NAFLD female patients present higher WHtR. The WHtR's accuracy in predicting NAFLD is acceptable compared to other currently suggested scores.

57ASM – 096 | Artificial Intelligence (AI) techniques and quantitative ultrasonography in nonalcoholic fatty liver disease

S. Santoro[1,2]; M. Khalil[1]; H. Abdallah[1]; I. Farella[1]; E. Lanza[1,3]; R. Abele[1]; L. Bonfrate[1]; A. Di Ciaula[1]; P. Portincasa[1]

[1]Clinica Medica "A.Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy; [2]Eurisko Technology Srl, Modugno, Italy; [3]Group of Psychiatric Neuroscience, Department of Basic Medical Science, Neuroscience and Sense Organs, Bari, Italy

Background: Ultrasonography (US) is frequently employed to diagnose nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease worldwide. However, the role of US in providing quantitative information about the grade of disease is still uncertain, also due to possible inter-observer variability.

Methods: 20 dysmetabolic patients (11 F, 9 M) underwent liver US (Sonoscape E2 Exp by Fuji-film Ultrasound machine provided by Eurisko Technology srl and equiped with 1–7 Mhz 3C-A convex probe setted at 3.5 Mhz) to grade the extent of fatty liver by US: grade 0 (normal liver, comparable liver/kidney cortex echogenicity); grade 1 (mild NAFLD, increased liver echogenicity); grade 2 (moderate–severe steatosis, markedly hyperechoic liver,

poor penetration of posterior seg-ments, poor visualization of hepatic veins and diaphragm). Hepatorenal index (HRI) and Attenuation Imaging Signal (ATI) were also recorded. We therefore checked the efficiency of Artificial intelligence (AI) aided techniques in the automatic assessment of NAFLD based on HRI calculation.

Results: Prevalence of steatosis was 55% in females (50% mild, 50% moderate), 78% in males (22% mild, 56% moderate, 22% severe). HRI evaluated by AI was significantly correlated with NAFLD grade ($r = 0.61$, $p = 0.004$), manual HRI assessment ($r = 0.54$, $p = 0.015$) and ATI ($r = 0.56$, $p = 0.0095$). AI-HRI adequately discriminated mild and moderate steatosis ($p = 0.04$). This was not the case using manual HRI assessment ($p = 0.09$). Neither AI-HRI nor manual HRI were able to discriminate moderate from severe NAFLD, probably due to low number of patients with severe steatosis (2 males, 10% of total).

Conclusion: Quantitative US represents a promising tool in the assessment of NAFLD. Although AI techniques might ameliorate the diagnostic accuracy of quantitative US, the use of commonly employed quantitative indices (i.e., HRI, ATI) still needs a full validation.

57ASM – 116 | Nonalcoholic fatty liver, adherence to Mediterranean diet and cardiometabolic risk

I. Galerati[1]; R. Abele[2]; M. Khalil[1]; A. Montesano[1]; H. Abdallah[1]; A. Di Ciaula[1]; M. Krawczik[2]; P. Portincasa[1]
[1]Univeristy of Bari, Bari, Italy; [2]Department of Medicine II, Saarland University Medical Center, Saarland University, Homburg, Germany,

Introduction: Nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease, has been linked with increased cardiovascular risk, and Mediterranean diet (MD) is emerging as a healthy habit, with multi-level beneficial effects. However, the links between the adherence to MD and the cardiometabolic risk in NAFLD patients are still under debate.

Methods: the adherence to MD was calculated (questionnaire) in 69 NAFLD patients (40.6% females, mean age: 54.3 ± 3.7 years, Body Mass Index [BMI]: $28.7 \pm 1.2\,kg/m^2$) with or without metabolic alterations (overweight/obesity 92%, diabetes 64% and/or dyslipidaemia 53%). Based on the final score (0–18) adherence was classified as low (score 0–6), intermediate (7–12) or high (13–18). Subjects also underwent blood tests and abdominal ultrasound to measure the extent of visceral and subcutaneous fat, and liver fibrosis by acoustic radiation force impulse shear wave elastography (ARFI). The CV risk was determined by ASCVD (Atherosclerotic Cardiovascular Disease Risk Calculator, American college of cardiology).

Results: Overall, the adherence to MD was intermediate (8.2 ± 1.2). Low adherence was recorded in 29% of patients. None reported high adherence. BMI and fat distribution were comparable in patients with low or moderate adherence. The grade of NAFLD and fibrosis were lower in patients with moderate- than in those with low adherence to MD ($p < 0.05$). Regarding blood parameters, only HDL concentration was higher in subjects with moderate, than in those with low adherence (47.8 ± 2.2 mg/dL vs. 35.3 ± 2.3 mg/dL, $p = 0.045$). Patient with moderate adherence to MD had a lower ASCVD than those with low adherence (12.2% vs. 20.5%, $p < 0.05$). The ASCVD score was higher in NAFLD patients with-, than in those without diabetes (19.4% vs. 9.4%).

Conclusions: Our clinical findings support the benefits of MD in terms of cardio-metabolic risk. Higher adherence to MD is linked with lower liver damage and cardiovascular risk in NAFLD patients.

57ASM – 159 | Metabolic remodelling in regenerating liver

B. Endaya[1,2]; L. Kučera[3]; T. Dan Diem Le[1]; Ms Gabriela Lopes Oliveira[1,4,5]; Prof Jiří Neužil[1,2,6,7]
[1]Institute of Biotechnology, Czech Academy Of Sciences, Vestec, Czech Republic; [2]First Faculty of Medicine, Charles University, Czech Republic; [3]Czech Centre for Phenogenomics, Institute of Molecular Genetics, Czech Academy of Sciences, Vestec, Czech Republic; [4]Center for Neuroscience and Cell Biology, University of Coimbra, Portugal; [5]Institute for Interdisciplinary Research, University of Coimbra, Portugal; [6]Faculty of Science, Charles University, Czech Republic; [7]School of Pharmacy and Medical Science, Griffith University, Southport, Australia

Introduction: Liver is unique in its ability to regenerate upon injury and tissue loss. This process of regeneration is extremely fast thereby inducing a rapid compensatory hyperplastic response to normalize lost functional capacity. To cater to this need for fast regeneration, the liver undergoes a highly coordinated series of metabolic 'switches' at different stages of regeneration to effectively 'shuttle and shuffle' metabolites into anabolic pathways. To date, many of these 'switches' remain undefined. Our aim is to identify the dynamic metabolic changes and how they are regulated to support rapid liver parenchymal reconstitution. Understanding this process may bring potential benefit to pathologies of liver with decreased capacity to regenerate.

Materials and Methods: We determined metabolic and phenotypic changes at different stages of liver regeneration after 40% hepatectomy, using in vivo molecular and histological imaging techniques, in combination with integrated targeted proteomics and spatial metabolomics (MALDI).

Results: Marked acute steatosis-like phenotype within 24 h of regeneration constituted an early hyperplastic response followed by exponential mitotic replication immediately post-steatosis, signifying lipids as a potential source to fuel regeneration and biomass synthesis. Interestingly, a metabolic switch in the urea cycle promoted a shift from ammonia detoxification to its diversion into metabolic pathways converting it to substrates for amino acids and de novo nucleotide syntheses.

Conclusion: To sustain the high level of proliferation in regenerating liver, metabolism switches to anabolic pathways coupled with metabolic recycling of ammonia to support rapid liver biomass synthesis.

57ASM – 195 | Artificial intelligence for diagnosis of hepatocellular carcinoma: Evidence-based synthesis

Azharuddin; M. Sharma

Department of Pharmacology, Jamia Hamdard, New Delhi, India

Background: Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death. Although the early detection and diagnosis are required to delay the disease worsening. In the last decade, the application of artificial intelligence (AI) has emerged in the field of medicine, and is now used for diagnostic, therapeutic and prognostic purposes in almost all fields. Therefore, this study aimed to assess the role of AI in diagnosis of hepatocellular carcinoma.

Material and Methods: A systematic literature search was performed on PubMed/Medline and Cochrane Library for the last 10 years using relevant pairing keywords. Google Scholar and open search were also conducted to search for grey literature. Eligible studies are identifying observational studies to assessing the role of AI in diagnosis of hepatocellular carcinoma. English language articles were retrieved, screened, and reviewed by the independent authors.

Results: A total of 16 studies were identified. Most of the studies ($n = 5$) were used computed tomography (CT) as diagnostic technique followed by ultrasound ($n = 4$), magnetic resonance imaging (I) ($n = 3$), histology ($n = 2$), and one each for contrast-enhanced ultrasound (C-US) and positron emission tomography (PET).

A convolutional neural network (CNN) was the commonly applied advanced AI technology. Five studies used deep learning as an AI tool, four studies used machine learning, and only one study used radiomics as AI tool. There is a variation in individual studies in terms of classification, labeling, training process, dataset size, and algorithm validation of AI. The included studies reported that, the AI models demonstrated superior diagnostic performance.

Conclusions and Recommendations: The current study suggested that AI model has a superior diagnostic performance. With the continued development of AI-assisted technologies, the application of these models in clinical practice supported by clinicians' experience and guided by patient-centered healthcare principles should be constantly considered in the future.

57ASM – 205 | Adherence to Mediterranean Diet, comorbidities, and MAFLD or NAFLD in Lebanese and Italian cohorts

N. Jaber[1]; H. Abdallah[1]; M. Khalil[1]; A. Zeaiter[2]; L. Mahdi[2]; A. Daher[2]; H. Shanmugam[1]; A. Di Ciaula[1]; P. Portincasa[1]

[1]Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy; [2]Rammal Rammal Laboratory, ATAC group, Faculty of sciences, Lebanese University, al-hadath campus, Beirut-Lebanon., Beirut, lebanon

Background: Several epidemiological and clinical studies show that Mediterranean diet (MD) can decrease cardiovascular and metabolic risk, including non-alcoholic- or metabolic associated- fatty liver disease (NAFLD/MAFLD). However, the existence of possible heterogeneous effects induced by both dietary habits and fatty liver subtype on the prevalence of comorbidities in people living in different Mediterranean areas is still scarcely explored.

Methods: Adherence to MD and presence of comorbidities were examined by questionnaires in patients with a previous diagnosis of fatty liver living in Italy and Lebanon. According to metabolic assessment and alcohol consumption, enrolled subjects were classified as NAFLD (No metabolic alterations with no significant alcohol consumption), NAFLD/MAFLD (metabolic alterations with no significant alcohol consumption), or MAFLD (metabolic alterations with significant alcohol consumption).

Results: Enrolled subjects were 49 Lebanese and 61 Italian. Italians were older than Lebanese subjects (48.6 ± 1.5 vs. 41.6 ± 2.0 yrs, respectively, $p = 0.006$). The

body mass index was comparable in the two groups (Italy 32.1 ± 0.7Kg/m2; Lebanon 31.1 ± 0.9Kg/m2, p = NS). Italian patients showed a higher adherence to MD (score 9.1 vs. 7.8 $p = 0.04$) and a lower prevalence of comorbidities (56% vs. 78%, $p = 0.01$) than Lebanese subjects. However, the prevalence of "pure" MAFLD was higher in the Italian (44%) than in Lebanese (2%, $P < 0.0001$). Conversely, Lebanese subjects showed a trend towards increased NAFLD prevalence (13% vs. 5% in Italians) and a significantly higher MAFLD/NAFLD prevalence (Italy: 51%; Lebanon: 85%; $p = 0.0002$).

Conclusions: In patients with fatty liver, the adherence to MD can be variable in people living in different Mediterranean areas. Irrespective of BMI, a higher prevalence of comorbidities can be linked with a low adherence to MD and with the presence of NAFLD or NAFLD/MAFLD, rather than of "pure" MAFLD. Further studies are needed to better disentangle the role of diet and different subtypes of fatty liver in determining the individual health risk.

SYMPOSIUM 5 – REGENERATIVE MEDICINE AND GENE/CELL THERAPY

57ASM – 044 | 3D bioprinting of multi-layered aortic-like constructs with a novel double-crosslinked decellularized ECM-bioink

F. Potere[1]; G. Venturelli[1]; B. Belgio[1]; F. Boschetti[1]; P. Petrini[2]; S. Mantero[1]
[1]Laboratory of Biological structure mechanics (LaBS)-Politecnico di Milano, Milano, Italy; [2]Politecnico di Milano, Milano, Italy

Introduction: Cardiovascular disease-related mortalities have risen from 12.1 million in 1990 to 18.6 million in 2019. 3D bioprinting technique has been exploited due to incomparable advantages to produce living tissues extruding hydrogels with embedded cells by layer-by-layer deposition of different cell types in the custom geometry of the construct. The study aim is to combine the advantages of decellularization process, 3D bioprinting, cells, and natural polymers to develop a novel bioink able to reproduce vessel substitutes compliant with shape and functionality requirements of the native tissues.

Material and Methods: Decellularization protocol was optimized to remove cellular and nuclear components from native tissues. Then, a decellularized powder was produced by cryomilling and solubilized with pepsin digestion. Several solutions, based on gelatin, alginate, and digested dECM, were studied to produce the bioink. A double-crosslinking process was optimized to obtain a stable structure after printing.

Results: The validation of the decellularization process was performed through DNA quantification, and appropriate staining. The optimized bioink was able to withstand the printing of a segment of tubular construct up to about 20 mm and to reproduce the multicellular complexity. The suspension resulting from 1.2% w/v gelatin, 6% w/v alginate, and 0.66% w/v dECM combined with a pre-printing crosslinking phase with 0.5% CaCO3 and 1.2% of GDL and a post-printing crosslinking with 1% CaCl2, was able to produce the desired structures. The dimensions of the printed structures are very close to those established in the design phase.

Conclusions: This novel bioink allows the printing of tubular structures capable of maintaining the desired structure, despite the increase in the number of layers. The parameters optimization allows the gain of structures characterized by a significant height of 40 layers. By tailoring the printing parameters and the amount of dECM, the desired mechanical properties can be met.

57ASM – 064 | Combined Baclofen with Pregabalin administration as a potential therapy for Spinal Cord Injury

M. F. Lima[1,2]; A. Mira[1,2]; A. G. Pinho[1,2]; D. J. Santos[1,2]; A. Barreira-Iglesias[3]; N. de Sousa[1,2]; A. J. Salgado[1,2]
[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; [2]ICVS/3B's Associate Lab, PT Government Associated Lab, Braga, Portugal; [3]Department of Functional Biology, CIBUS, Faculty of Biology, Universidade de Santiago de Compostela, Braga, Portugal

Traumatic spinal cord injury (SCI) is a serious damages that occurs in central nervous system (CNS), resulting in motor and sensory functional impairment. Besides these functional alterations, spasticity and neuropathic pain affect most injured patients, having a significant impact on their quality of life.

Previous data showed that baclofen administration at 1 mg/Kg in the acute phase after injury, produces a significant improvement in locomotor behaviour, increases bladder control and modulates the immune response in a spinal cord injury rodent model. Pregabalin is a drug used to manage neuropathic pain in patients with spinal cord injury. Furthermore, recent studies have shown that pregabalin has a neuroprotective action after spinal cord injury. Combined administrations of baclofen with pregabalin, two GABAergic drugs, may result in a potential effect that

could promote functional and neurological improvements in patients with spinal cord injury. Therefore, we propose to study this combinatory medical approach using a SCI rodent model to understand its therapeutic potential in the spinal cord injury field. Our aim goal to provide significant insights that could potentially have profound implications in SCI patients.

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57ASM – 079 | An improved radiolabeling strategy of small extracellular vesicles (sEVs) employing 89Zr4+ for prolonged in vivo monitoring using PET imaging

A. Banerjee[1]; I. Hrynchak[2]; K. Simon[1]; F. Tejo[4]; Q. Ribeiro[1]; A. Abrunhosa[2]; L. Ferreira[1,3]

[1]CNC-Center for Neurosciences and Cell Biology, University of CoimbraCoimbra, Portugal; [2]ICNAS – Institute for Nuclear Sciences Applied to Health; University of Coimbra; Pólo das Ciências da Saúde, Azinhaga de Santa Comba, Coimbra, Portugal; [3]Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [4]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

sEVs are biological nanoparticles, with sizes between 30 to 200 nm, secreted by most cells1. The market for sEV-based diagnostics and therapies has grown over the years, necessitating the use of PET imaging to monitor sEVs for extended periods of time. 89Zr4 + has a higher half-life of 78.4 h with a relatively low positron energy of 395.5 keV, cheaper to produce makes it a favourable choice for PET imaging2. The most common metal chelator for 89Zr4+ is deferoxamine (DFO) which forms the 89Zr4 + −complex very fast at room temperature3. However, several preclinical animal models show the leaching of 89Zr4+ from the DFO-89Zr4+ after 1 h of administration4. Alternatively, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) forms a stable 89Zr4 + −complex at high temperature (90°C).

Our aim was to develop a chelator for 89Zr4+ which can form a stable complex at room temperature and use it for PET imaging. The chelator was able to complex 89Zr4+ in 1 h. The highest complexation was observed in Tris-buffer. Radiolabelling of the sEVs was done by a two-step process where in the first step the chelator was conjugated with the sEVs and in the next step the radiolabelling was done using 89Zr4+. sEV-chelator-Zr had a radio-chemical yield of ~60% and ~80% stability in plasma for 7 days. Our modification did not affect the morphology, surface protein and internal RNA content of sEVs. Finally, we successfully imaged sEVs in C57BL/6J mice after injecting sEV-chelator-89Zr intravenously and measured the bio-distribution of it in different organs for a prolonged time.

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57ASM – 087 | Secretome of adipose tissue derived stem cells and electrical epidural stimulation promotes functional gains in spinal cord injury context

J. Ribeiro[1,2,3]; D. Silva[1,2]; T. Pinho[1,2]; S. Serra[1,2]; N. Silva[1,2]; R. Ichiyama[3]; A. Salgado[1,2]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3]Faculty of Biological Sciences, University of Leeds, Leeds, UK

Background: Spinal Cord Injury (SCI) is a life changing event with a high number of cases reported every year, causing motor and sensorial disfunction to the patients. Adipose Stem cells (ASCs) secretome was reported to promote locomotor improvements in a mice model of SCI, due to neuroprotectants, growth factors and immunomodulators present on it. The aim of this work was to combine this potential with electrical epidural stimulation (EES), know to promote spinal plasticity, central pattern generator activation and steeping initiation, to obtain higher functional gains.

Materials and Methods: Spinal cord slices were used for the in vitro experiments. Slices were growth and histological analysis performed at day 7. Studies involving electrical stimulation in vitro were conducted in a mixed culture of spinal cord cells. Female rats (30) were divided into four groups: Control, Secretome, Secretome+EES, Secretome+EES + Training. Locomotor and stepping analysis were performed 2 months after SCI.

Results: We observed an increase of neurite outgrowth and migration of iba-1 positive cells facilitated by the secretome ($p < 0.05$). Using a TrkB approach, we detected this is mediated in part by BDNF. Secretome also protects the spinal cord cells after a glutamate insult and reduces astrogliosis in SCI slices. We also show that EES promotes

different outcomes on the spinal cord cells depending on the stimulation settings. Lastly, the combinatory approach of secretome and EES in a rat model of SCI in vivo, improves locomotor performance, body weight support, maximum speed, number of steps and dragging time during the stepping cycle after SCI.

Conclusions: This provides evidence of the therapeutic potential of ASCs secretome and EES after SCI, supported by the positive effects exerted on neuroinflammation and axonal outgrowth in vitro. This potential is highlighted with the combinatory approach in vivo, with functional gains observed in the locomotor performance when compared to control.

57ASM – 114 | Targeted delivery of therapeutic agents to the heart using click chemistry

Q. Ribeiro[1]; A. Vilaça[1]; A. Abrunhosa[2]; A. Banerjee[1]; L. Ferreira[1,3]

[1]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; [2]Institute for Nuclear Sciences Applied to Health, University of Coimbra; Pólo das Ciências da Saúde, Azinhaga de Santa Comba, Coimbra, Portugal; [3]Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Introduction: Cardiovascular disease is the main cause of mortality worldwide. Although there are several available therapies to address this issue, targeting the heart remains a challenge. Improving targeting strategies is key to unravel the regenerative potential of drugs. The bio-orthogonal click reaction between functionalized dibenzocyclooctyne (DBCO) and azide-containing sugars, incorporated on the cell surface by metabolic labelling, holds great potential as a rapid, efficient, and specific targeting strategy. Among the described azido sugars, we aim to determine the best one for cardiac metabolic labelling, which will improve cargo retention in the endothelial barrier by click chemistry.

Materials and Methods: Primary human endothelial cells (HUVECs) were incubated with different concentrations of azido sugars (5-50uM) – N-azidoacetylmannosamine-tetraacylated (Ac4ManNAz), N-azidoacetylgalactosamine-tetraacylated (Ac4GalNAz), or N-azidoacetylglucosamine-tetraacylated (Ac4GlcNAz) – to promote metabolic labelling. After incubation, cells were stained with Cyanine5 DBCO for the detection of azide groups. Finally, we evaluated the internalization of a DBCO-coupled nanoformulation in the presence of sugars. Data were analysed with one-way ANOVA followed by Tukey's comparison test, in GraphPad Prism software.

Results: Our results demonstrate that, from the three sugar derivatives used, the fluorescence intensity per area of Cyanine5 DBCO in cells was higher with Ac4GlcNAz. These azide-surface modified cells were able to internalize higher levels of DBCO-functionalized cargo than cells without azide modification.

Conclusion: We observed that Ac4GlcNAz is the most expressed azido sugar upon metabolic labelling of cells. Moreover, the nanoparticles, containing DBCO, are able to react with the cell surface azide and being internalized. We have, therefore, identified a simple but very promising strategy to target endothelial cells using click chemistry. We are now exploring this approach to label cardiac endothelial cells in an in vivo setting.

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57ASM – 115 | GABA system activation immunomodulatory role after spinal cord injury

A. Correia-Silva[1,2]; A. G. Pinho[1,2]; H. Cavaleiro[1,2]; J. R. Cibrão[1,2]; D. J. Santos[1,2]; M. F. Lima[1,2]; T. G. Pinho[1,2]; S. Monteiro[1,2]; A. Barreiro-Iglesias[3]; A. J. Salgado[1,2]; N. de Sousa[1,2]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; [2]ICVS/3B's – PT Government Associate Laboratory, Braga, Portugal; [3]Department of Functional Biology, CIBUS, Faculty of Biology, 15782 University of Santiago de Compostela, Santiago de Compostela, Spain

Introduction: Spinal cord injury (SCI) is a devastating neurological condition that results in loss of motor and sensory function below the injury site. Baclofen is a GABA agonist widely used to manage spasticity, particularly on chronic SCI patients. Our hypothesis is that GABA system activation through Baclofen could minimize the secondary injury, through the modulation of the immune response, providing a permissive environment for SCI recovery. The current knowledge in the field, revealing an emerging role of GABA in the modulation of neuroinflammation, strongly supports the proposed hypothesis. Here, we compare the administration effects of different Baclofen doses (1 and 5 mg/kg) in a SCI in vivo model.

Materials and Methods: Adult female C57BL/6J mice, 8–12 weeks old, were used to establish a SCI in vivo model. The injury was performed at T8 level by a 10 second compression using forceps. Within the first 5 min post-injury animals were treated (by intraperitoneal injection) with Baclofen at 1 or 5 mg/kg, and saline was used as control.

At 1, 7 and 14 days-post-injury we profiled the immune response using local (spinal cord), systemic (blood) and peripheric (spleen and thymus) samples by flow cytometry. This timepoints are major landmarks in the immune response after SCI, being characterized as peaks of neutrophils, monocytes, and lymphocytes at the injury level, respectively. Additionally, a commercial kit for cytokine detection was used with serum samples collected in the same timepoints.

Results: Our preliminary findings suggests that acute Baclofen administration after SCI can modulate the immune response, namely affecting lymphoid lineage cell infiltration at the injury site.

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57ASM – 141 | Endothelial conditioned media induce Tissue Factor expression and angiogenic activity in human adipose-derived stem cells

A. Civit Urgell[1]; L. Badimon[2]; G. Arderiu[2]

[1]Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, Barcelona, Spain; [2]Ciber CV, Instituto Carlos III, Madrid, Spain

Introduction: The adipose-derived stem cells (ASCs) have been considered as a desirable source of cells for cell therapy. ASCs have the capacity of differentiating into endothelial cells (ECs) and initiate the angiogenic process. Because it is known that tissue factor (TF) is highly expressed in ASCs, the aim of the study was to investigate the role of the TF signalling pathway in ASCs-mediated angiogenesis.

Materials and Methods: ASCs were cultured with growth factors, such as transforming growth factor (TGF)-β1 or basic-fibroblast growth factor (bFGF), or EC-conditioned medium (EC-CM) for 14 days. Protein levels and NA expression were used to analyse the regulation of the ASCs-TF axis. TF subcellular localization in ASCs and the activated signalling pathways were analysed.

Results: ASCs expressed higher levels of TF than ECs. The different ASCs growth condition used to promote differentiation into ECs affected the expression levels of TF. ASCs cultured with EC-CM showed an up-regulation of TF NA expression and protein levels, that was sustained during all the differentiation process (from day three until day fourteen) ($p < 0.01$). On the other hand, in ASCs cultured with TGF-β1 and bFGF we observed a progressively decrease in TF protein levels and NA expression. While, TGF-β1 showed a trend of TF appearance in the cytoplasm, bFGF showed a clear decrease of TF in the cytoplasm. These TF subcellular location changes were dependent on culture condition. ASCs cultured with EC-CM or TGF-β1 showed a stimulated TF signalling pathway with up-regulated ETS1 expression and inhibited SMAD3. Conversely, ASCs treated with bFGF resulted in an inhibition of ETS1 and upregulation of SMAD3.

Conclusions: Our results indicate that TF expression in ASCs is highly regulated. It is modulated by growth factors and culture conditions and affects several signalling pathways.

57ASM – 180 | Secretome of physiological primed mesenchymal stem cells modulate common survival pathways in a in vitro HIE model

I. Caramelo[1,2]; V. M. Mendes[1]; I. L. Salazar[1,3]; C. Cardoso[4]; A. Dinis[5]; C. B. Duarte[6]; M. Grãos[7]; B. Manadas[1,3]

[1]CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Cantanhede, Portugal; [2]PhD Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Casa Costa Alemão, Coimbra, Portugal, [3]Institute for Interdisciplinary Research, University of Coimbra (IIIUC), Coimbra, Portugal; [4]Crioestaminal, Cantanhede, Portugal; [5]Pediatric Intensive Care Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [6]Faculty of Sciences and Technology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal; [7]Biocant, Technology Transfer Association, Cantanhede, Portugal

Introduction: Perinatal asphyxia is caused by an insufficient blood supply to the neonate in the peripartum period, usually leading to hypoxic–ischemic encephalopathy (HIE), a brain injury that usually results in profound neurophysiological sequelae or even death. Currently, therapeutic hypothermia is the standard of care but is focused on preventing the expansion of the damage rather than promoting neuronal repair. Therefore, umbilical cord mesenchymal stem cells (UC-MSCs) secretome is emerging as a promising therapeutic approach. Recent studies demonstrated that these cells are capable of secreting a wide range of molecules that, through paracrine effects, trigger different pathways. However, an extensive expansion in vitro might compromise MSCs' stemness. Our group previously demonstrated that culturing MSCs in environments similar to the umbilical cord – controlled

stiffness (≈3 kPa) or oxygen levels (≈5%O_2) – could modulate MSCs secretome when compared to "standard" culture conditions (plastic (≈1GPa), at ≈18%O_2). We hypothesized that these differences might have different effects on neurons.

Methods: Primary rat neurons were subjected to oxygen and glucose deprivation (OGD) stimuli at DIV8. Then, neurons were incubated with the secretome of primed-MSCs, and the cellular proteome was quantified using mass-spectrometry. Proteins quantified with confidence were then subjected to a univariate and multivariate analysis to identify molecules that were altered in the presence of MSCs secretome.

Results: More than 150 proteins were found to be commonly modulated by the three secretomes and are mainly involved in microtubule organization and axiogenesis. In addition, we identified more than 200 proteins that were significantly different from OGD in the presence of the secretome but did not vary compared to sham. These proteins are mostly associated with ribosomes and involved in translation processes, such as L13a-mediated translational silencing of ceruloplasmin.

Conclusion: Our data revealed important pathways for neuronal survival that could be used as a new drug target.

57ASM – 181 | Unravelling the secretome of MSCs cultured at physiological conditions: A proteomics screening

I. Caramelo[1,2]; V. M. Mendes[1]; C. Domingues[1,3]; A. C. Santo Mendes[1,2,4]; M. Geraldo[1],. S. I. Anjo[1]; C. Cardoso[5]; A. Dinis[6]; C. B. Duarte[7]; M. Grãos[4]; B. Manadas[1,3]

[1]CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; [2]PhD Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Casa Costa Alemão, Coimbra, Portugal; [3]Institute for Interdisciplinary Research, University of Coimbra (IIIUC), Coimbra, Portugal; [4]Biocant, Technology Transfer Association, Cantanhede, Portugal; [5]Crioestaminal, Cantanhede, Portugal; [6]Pediatric Intensive Care Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; [7]Faculty of Sciences and Technology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Introduction: Mesenchymal stem cells (MSCs) are emerging as a promising therapeutic approach in several diseases since they can migrate to the lesion upon injection. Particularly, in ischemic brain injury, they are known to modulate inflammation and promote neuroregeneration. Recent studies have described that the beneficial effects are mainly through paracrine effects, that in response to the environment they sense at the injury site, MSCs are able to secrete molecules and modulate the response of neighbour cells. However, obtaining a "single dose" of MSCs secretome requires extensive in vitro expansion, which might compromise their stemness. Therefore, we hypothesized that culturing MSCs in a more physiological environment could modulate their secretome.

Methods: Umbilical-cord MSCs (UC-MSCs) were cultured on platforms with controlled conditions, mimicking the stiffness (≈3 kPa), or oxygen levels (≈5%O_2) that they sense on the umbilical cord until P4. The secretome was collected, concentrated, analysed by mass spectrometry and compared to "standard" culture conditions (plastic (≈1GPa), at ≈18%O_2). Then, a multivariate analysis was performed using the proteins quantified with confidence. For each condition (control vs. mechanomodulation or physoxia), a Partial Least-Squares Discriminant Analysis was built, and proteins with a VIP >1 were selected to further analysis.

Results: Gene ontology analysis revealed that cytoplasmic and mitochondrial proteins, as well as energy pathways and translation processes, are modulated by stiffness. On the other hand, MSCs cultured at physiological oxygen levels differentially secrete proteins involved in the immune response and with protease inhibitor activity. Interestingly, more than fifty proteins are commonly altered on both physiological stimuli and are mostly associated with protein metabolism, exosomes, and lysosomes.

Conclusion: Taken together, these data suggest that physiological culture conditions can modulate the secretome in similar ways. We hypothesize that modulation of the secretome will empower their therapeutic effects on brain injury.

57ASM – 220 | Nanoformulation with spatio-temporal control for brain gene editing

S. Simões[1]; M. Lino[1]; A. Barrera[1]; F. Tomatis[2]; R. González[2]; A. Carvalho[2]; A. Neuner[3]; S. Sricker[3]; L. Ferreira[4]

[1]University of Coimbra, Coimbra, Portugal; [2]Center for Neuroscience and Cell Biology, Coimbra, Portugal; [3]Epigenetic Engineering, Institute of Stem Cell Research, Helmholtz Zentrum, Munich, Germany; [4]Faculty of Medicine of University of Coimbra, Coimbra, Portugal

Introduction: The CRISPR/Cas9 system has emerged as a promising platform for gene editing however the lack of an efficient and safe delivery system into cells. Unfortunately, viral delivery methods of CRISPR-Cas9

may cause genomic undesirable side effects because of the prolonged expression of the CRISPR system. In addition, they do not offer spatial resolution to allow an extra-level of safety. Here, we propose a formulation that has the capacity to release intracellularly the Cas9/sgRNA ribonucleoprotein complex with remote control, using near-infrared-light as a trigger.

Methods: For proof of concept, the gene editing was evaluated by the eGFP knockout in d2eGFP-HeLa cells. Then, we evaluated the in vitro gene editing in different brain cell populations (subventricular zone [SVZ] cells, astrocytes and neuros). The different brain cell populations were isolated from a transgenic mice Ai9 with a cassette inserted into the Gt(ROSA)26Sor locus with a LoxP-Stop-LoxP-tdTomato-sequence. To demonstrate in vivo gene editing, AuNRs were stereotaxically injected into the SVZ of both hemispheres of adult Ai9 brain mice. The gene editing expression was evaluated two weeks after.

Results: D2eGFP-HeLa cells treated with the formulations and with light-activation showed more than 70% of eGFP knockout activity. The gene editing can be initiated upon 24 h after cell transfection demonstrating a temporal control. In the brain cell population, the gene editing efficiency was determined by the quantification of tdTomato-positive cells. The formulation was able to edit SVZ cells, astrocytes and neurons. In in vivo the expression of tdTomato fluorescence was higher in irradiated hemisphere.

Conclusion: We have developed a gene-editing formulation for on-demand release into deep tissues with spatial-resolution upon exposure to transcranial NIR light.

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57ASM – 222 | Engineered extracellular vesicles for the treatment of ischemic diseases

M. Lino[1,2]; L. Nicastro[3]; F. Catapano[3]; D. Hayman[3]; I. Kostic[4,5]; C. Terracciano[3]; C. Emanueli[3]; L. Ferreira[1,2]

[1]Faculty of Medicine of the University of Coimbra, Coimbra, Portugal; [2]CIBB-Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; [3]National Heart & Lung Institute, Imperial College London, Hammersmith Campus, ICTEM, London, UK; [4]Division of Bioengineering in Medicine, Department of Medicine, Center for Regenerative Therapeutics, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, USA; [5]Harvard Stem Cell Institute, Cambridge, MA, USA

Introduction: Therapeutic approaches currently implemented in the clinic to treat myocardial infarction enable restoration of blood flow but do not provide regenerative capability to the heart. Extracellular vesicles (EVs), i. e. nano-sized vesicles secreted by cells, containing a cocktail of bioactive molecules, have emerged as an alternative therapy for this acute pathology. Some studies have demonstrated the regenerative potential of EVs from different sources (such as mesenchymal stem cells (MSCs) and cardiac progenitor cells), either in their native form or engineered [1]. Specifically, targeting strategies have been explored with the aim of enhancing the effect of EVs by increasing their accumulation in the ischemic region after systemic administration.

Materials and Methods: Herein, we developed a strategy that goes beyond the current targeting approaches by chemically modifying the surface of EVs secreted by human adipose derived MSCs with a peptide that works both as targeting and therapeutic/bioactive agent. The peptide targets a lectin highly expressed in the heart after myocardial infarction and involved in the progression of inflammation and fibrosis [2, 3].

Results: Our data indicates that EVs can be conjugated with controllable amounts of peptide. Additionally, engineered EVs are able to accumulate more in endothelial cells leading to increased angiogenic and anti-apoptotic activity, as well as in living myocardial slices exposed to hypoxic conditions, restoring their contractile properties.

Conclusion: In this work we developed an EV-based formulation with pro-regenerative activity for the treatment of heart ischemia.

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References

1. de Abreu, R. C. et al. Nature reviews. Cardiology 2020
2. Hashmi, S.; Al-Salam, S. Cardiovascular pathology: the official journal of the Society for Cardiovascular Pathology 2015
3. Morrow, D. A.; O'Donoghue, M. L. Journal of the American College of Cardiology 2012.

57ASM – 232 | Synergistic antimicrobial effect between antimicrobial peptides conjugated nanoparticles

A. Rai[1]; D. Cruz[1]; L. Ferreira[1,2]
[1]*CNC - Center for Neuroscience and Cell Biology, CIBB-Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, UC, Biotech Parque Tecnológico de Cantanhede, 3060-197 Coimbra, Portugal;* [2]*Faculty of Medicine, University Coimbra, 3000-548 Coimbra, Portugal*

Antimicrobial peptides (AMPs) conjugated on the surface of nanoparticles (NPs) have attracted great attention to treat microbial infections [1]. These AMP-NPs have variable minimal inhibitory concentration (MIC), being very selective for bacteria species. Interestingly, some of AMP-NPs have also pro-regenerative activity but, in general, less powerful antimicrobial activity. We envision that the combination of two types of AMP-NPs having potent antimicrobial activity and regenerative property could be an interesting approach to treat infections. Here, we demonstrate the antimicrobial synergistic effect of two AMP-conjugated gold NPs (Au NPs) in killing Gram-positive and Gram-negative bacteria. Cecropin-melittin-conjugated Au NPs (CM-Au NPs; high antimicrobial activity but low or absent pro-regenerative activity) and LL37-conjugated Au NPs (high pro-regenerative activity but low antimicrobial activity) were prepared using a simple one step synthesis approach [2,3]. The minimum inhibitory concentration (MIC) of CM-Au NPs is 40 mg/mL while MIC of LL37-Au NPs is 60 mg/mL against Gram-negative and Gram-positive bacteria. However, these MICs induce cytotoxic effect to human cells [3]. On the other hands, the combinations of different concentrations of CM-Au NPs and LL37-Au NPs (below the MICs) effectively kill bacteria. Importantly, ½ MIC of CM-Au NPs and ¾ MIC of LL37-Au NPs have synergistic antimicrobial effect to kill *E. coli* and *S. aureus* bacteria. This combination of both NPs rapidly permeabilizes the outer membrane compared to the inner membrane of genetically engineered *E. coli* ML35p bacteria. The kinetics of permeability is similar to the MICs of individual CM-Au NPs or LL37-Au NPs. Scanning electron microscopy analyses indicate the prominent damages in the membrane of *E. coli* and *S. aureus* bacteria. Overall, these results indicate that the combination of different AMPs conjugated NPs could be an alternative choice to potently kill bacteria while promoting skin regeneration.

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References:

1. A. Rai, R. ferrão, P. Palma, T. Patricio, P. Parreira, E. Anes, C. Tonda-Turo, C. Martins, N. Alves, L. Ferreira, J. Mater. Chem. 10, 2022, 2384.
2. M. Comune, A. Rai, K.C. Chereddy, S. Pinto, S. Aday, A. F. Ferreira, R. Cunha, R. Rodrigues, J. Lerma, P.N. Simoes, V. Preat, L. Ferreira, J. Cont. Release. 262, 2017, 58.
3. M. Comune, A. Rai, P. Palma, C. Tonda-Turo, L. Ferreira, Biomater. Sci. 9, 2021, 8153.

57ASM – 228 | Initiation of embryonic regeneration studied in time and space

R. Sindelka; J. Netusil; D. Kraus; P. Abaffy; M. Kubista
Institute of Biotechnology, Vestec, Czech Republic

Regeneration is an interesting biological process that occurs in invertebrates, fishes, reptiles and amphibians. Mammals also demonstrate regenerative capabilities, but it is soon lost after birth. Recently, we have made some advancements and revealed a novel cell population active during the early phase of regeneration. We named it "Regeneration Initiation Cells" (RIC) and studied in details genes specifically expressed in RICs. We will present their functions during tail regeneration in *Xenopus laevis* embryos determined by bulk, single cell RNA-Seq and spatial transcriptomics (10x Genomics) in combination with functional validation experiments. We believe that our results will serve as a backbone for a more comprehensive understanding of this fascinating process and aid in the future identification of promising therapeutic targets.

57ASM – 239 | Therapeutic application of sertoli cells in the restoration of male fertility after testicular inflammation

M. Krulová

Department of Cell Biology, Faculty of Science, Charles University, Czech Republic

Cell therapies represent an important development in human medicine; stem cells are the prominent tool for such therapies. Sertoli cells (SCs) are the most important contributor to spermatogonial stem cell niches, forming a blood-testis barrier but also producing a variety of biomolecules to support germ cell development and creating an immunosuppressive environment necessary for germ cell survival. Additionally, SCs can be easily isolated from patient testicular biopsies performed routinely in fertility clinics. Currently, we have confirmed that SCs possess properties of mesenchymal stem cells, including migration of SCs into damaged testes, paracrine and contact-dependent action and donation of mitochondria to various recipient cells. Therefore, we aimed to introduce Sertoli cell (SC) therapy to protect testicular immune privilege, suppress inflammation and support spermatogonial cells in males suffering from infections associated with the risk of impaired reproductive health. In an in vivo model of testicular inflammation, SC application suppressed immune cell infiltration and protected developing germ cells. Thus, the introduction of a new therapeutic approach using SC to address the cause of male infertility may help patients suffering from inflammatory testicular diseases.

57ASM – 241 | The effect of anticoagulants on stem cell properties of human bone marrow mesenchymal stem cells

M. Ferencakova[1]; A. Benova[1]; I. Raska[2]; P. Abaffy[3]; R. Sindelka[3]; M. Dzubanova[1]; E. Pospisilova[4]; K. Kolostova[4]; V. Zikan[2]; M. Tencerova[1]

[1]Laboratory of Molecular Physiology of Bone, Institute of Physiology of the Czech Academy of Sciences, Prague 142 20, Czech Republic; [2]Third Department of Medicine - Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic; [3]Laboratory of Gene Expression, Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czechia; [4]Laboratory of Personalized Medicine, Oncology Clinic, University Hospital Kralovske Vinohrady, Srobarova 50, 10034, Prague, Czech Republic

Background and Purpose: Bone marrow mesenchymal stem cells (BM-MSCs) are source of multipotent stem cells important for tissue engineering, regenerative medicine and diagnosis of bone diseases in patients. The isolation of human BM-MSCs from bone marrow (BM) cavity using BM aspiration applies the method with collection into tubes containing anticoagulants to avoid coagulation of BM aspirate. However, it is not known how exactly the processing of BM samples and interaction with anticoagulants may affect stem cell characteristics and composition of isolated BM-MSCs in the culture. Thus, we investigated whether a type of anticoagulant in BM-MSC isolation procedure affects their cellular and molecular characteristics.
Methods: Donors of BM ($n = 12$ (6F/6M), age, 48–85 years old) were recruited from hematologic clinic. BM aspirates were obtained from iliac crest and divided into tubes coated with different anticoagulant EDTA or Heparin to establish in vitro cell cultures of BM-MSCs. The paired samples of BM-MSCs were evaluated for cellular and molecular characteristics. Immunophenotypization by flow cytometry and RNA seq analysis was performed. Differentiation potential of BM-MSCs was determined by measurement of alkaline phosphatase (ALP) activity, Alizarin red staining for mineralized matrix formation and Oil-Red O staining for mature adipocytes.
Results: The paired samples of isolated BM-MSCs from Heparin and EDTA-coated tubes obtained from the same patient showed increased cellular yield in Heparin vs EDTA samples, accompanied with increased number of colony forming units-fibroblast (CFUs-f) numbers. Besides these findings, there were no significant changes in differentiation potential, immunophenotyping and molecular analysis by RNA seq between Heparin- and EDTA-isolated BM-MSCs. Interestingly, RNA seq analysis

revealed more significant changes between noncultivated and cultivated BM-MSCs regardless of used anticoagulant. Gene-ontology-enrichment analysis identified category of genes involved in nucleotide metabolism, cellular metabolism with higher expression, while genes involved in inflammation, chromatin remodelling were decreased in cultivated BM-MSCs.

Conclusion: Our study showed that a type of anticoagulant in BM-MSC isolation did not have an impact on molecular characteristics and cellular composition, while cultivation caused the major change in gene expression profile of BM-MSC, which is important information for further strategy using BM-MSCs in regenerative medicine and clinical therapy.

Key words: human bone marrow mesenchymal stem cells, anticoagulants, cultivation, stem cell characteristics, differentiation potential.

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SYMPOSIUM 6 – REPRODUCTIVE BIOLOGY

57ASM – 049 | Impact of the endocrine disruptor atrazine on the nutritional support of spermatogenesis

D. Gomes-Andrade[1,2]; B. Guerra-Carvalho[2,3,4,5,6]; D F. Carrageta[2,3,6]; R. L. Bernardino[2,3]; P. F. Oliveira[4]; M. de Lourdes Pereira[1,7]; M. G. Alves[2,3,6]

[1]Department Of Medical Sciences, University of Aveiro, Aveiro, Portugal; [2]Clinical and Experimental Endocrinology, Unit for Multidisciplinary Research in Biomedicine (UMIB), School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal; [3]Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, Porto, Portugal; [4]LAQV – REQUIMTE and Department of Chemistry, University of Aveiro, Aveiro, Portugal; [5]Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal; [6]Laboratory of Physiology, Department of Imuno-Physiology and Pharmacology, School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal; [7]CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal

Background: The endocrine disruptor atrazine regularly contaminates potable water supplies. Atrazine causes a variety of negative health outcomes, including changes in the neuroendocrine system and infertility. Within the testis, the mechanical and nutritional support of spermatogenesis is provided by Sertoli cells. Notably, the effects of atrazine on Sertoli cells metabolism remain unknown. In this work, we aimed to elucidate the effects of atrazine on the nutritional support of spermatogenesis by studying how the metabolic and mitochondrial function of Sertoli cells are affected.

Materials and Methods: Mouse Sertoli cells (mSCs) (TM4 cell line, $n = 10$) were exposed to biologically relevant concentrations of atrazine (in μg/L: 0.3, 3, 30, 300 and 3000). After 24 h, cytotoxicity was determined. Mitochondrial activity and total ROS production were measured using JC-1 dye and CM-H2DCFDA probe, respectively. FRAP assay was used to measure the antioxidant potential of culture media. Lactate dehydrogenase (LDH) protein levels were evaluated by Western Blot and glycolytic function by Seahorse XF Glycolysis Stress Test Kit. Data were analysed using RM one-way ANOVA.

Results: Exposure to atrazine (300 and 3000 μg/L) reduced the metabolic activity of mSCs, without causing cytotoxicity nor affecting cellular proliferation. In addition, exposure to atrazine caused a significant decrease in glycolysis, glycolytic capacity, and non-glycolytic acidification of mSCs. Concurrently, LDH expression in those cells was also decreased in a dose-dependent manner. Finally, we detected a tendency for both an increase in ROS production and a decrease in the antioxidant potential of the culture media of mSC after exposure to the highest atrazine concentrations.

Conclusions: Atrazine impairs mSCs glycolytic metabolism, potentially affecting lactate production, and creates a pro-oxidant state. Since lactate is the preferential energy substrate for germ cells and they are highly susceptible to oxidative stress, exposure to atrazine affects male fertility by impacting the nutritional support of spermatogenesis.

57ASM – 065 | AQP7-mediated glycerol permeability is impaired in spermatozoa from asthenozoospermic men

J. C. Ribeiro[1,2,3]; R. L. Bernardino[1,2]; A. Barros[4,5,6]; M. G. Alves[1,2]; P. F. Oliveira[3]

[1]UMIB – Unidade Multidisciplinar de Investigação Biomédica, ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; [2]Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, Porto, Portugal; [3]LAQV-REQUIMTE and Department of Chemistry, University of Aveiro, Aveiro, Portugal; [4]Centre for Reproductive Genetics Prof. Alberto Barros, Porto, Portugal; [5]Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal; [6]i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

Background: The bioenergetic mechanisms that allow sperm motility remains a mystery. Evidence suggests that glycerol is relevant during sperm maturation, where sperm cells acquire motility. Herein, we set to study the role of glycerol on sperm function and glycerol permeability's relation with sperm motility.

Methods: First, we confirmed the expression and localization of glycerol kinase 2 (GK2) and glycerol-3-phosphate dehydrogenase 2 (GPD2) in human sperm and compared its expression levels between the sperm of normozoospermic men (normal motility) and asthenozoospermic men (subnormal motility). Spermatozoa from normozoospermic men ($N = 30$) were collected and incubated with glycerol (100 μM), glucose (11 mM), glycerol plus glucose, or in the absence of carbon source for 6 h to measure its effect on sperm motility. High-motility sperm and low-motility sperm from fertile men were isolated by gradient centrifugation and their glycerol permeability was measured by stopped-flow light scattering. Sperm from normozoospermic men and asthenozoospermic men ($N = 30$) was incubated with or without specific aquaporin (AQP)3 (Z433927330), AQP7 (DFP00173), and an unspecific AQP inhibitor (phloretin), for 10 min to understand the importance of each AQP on sperm glycerol permeability and potential differences between fertile and subfertile men.

Results: Our results show that GK2 was expressed in the midpiece, whereas GDP2 was in the acrosome and the axoneme. GK2 expression was upregulated in spermatozoa from asthenozoospermic samples, which could point to the metabolic inefficiency characteristic of the spermatozoa from these samples. Incubation with glycerol helped maintain sperm motility. Glycerol plus glucose was better at preserving sperm motility than the groups of glucose and glycerol separately. AQP7 was responsible for glycerol diffusion, which mechanism was impaired in asthenozoospermic samples.

Conclusions: AQP7-mediated glycerol permeability is impaired in samples with low motility, and glycerol metabolization can be a source of NAD+ for sperm glycolysis, a process that is crucial for sperm motility.

57ASM – 070 | Epididymosome 3′mt-tiRNA-Met responds to high-fat diet and is negatively correlated with sperm quality

S. C. Pereira[1,2,3,4,5]; L. Freire-Brito[1,2]; B. Guerra-Carvalho[1,2,3,4,5]; D. F. Carrageta[1,2,5]; R. L. Bernardino[1,2]; L. Crisóstomo[6]; S. Terzo[7]; M. P. Monteiro[1,2]; P. F. Oliveira[3]; M. Allegra[7]; M. G. Alves[1,2,5]

[1]Endocrine and Metabolic Research, Unit for Multidisciplinary Research in Biomedicine, School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Portugal; [2]Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, Portugal; [3]LAQV-REQUIMTE and Department of Chemistry, Campus Universitario de Santiago, University of Aveiro, Portugal; [4]Department of Pathology, Faculty of Medicine, University of Porto, Portugal; [5]Laboratory of Physiology, Department of Imuno-Physiology and Pharmacology, School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Portugal; [6]Institute of Biomedicine, University of Turku, Finland; [7]Department of Pharmaceutical, Toxicological and Biological Chemistry, University of Palermo, Italy

Introduction: During the epididymal transit, spermatozoa are enriched in metabolic (epi)genetic cues through extracellular vesicles, particularly those coming from the principal cells (epididymosomes). Herein, we studied if high-fat diet (HFD) alters the integration of specific mitochondrial tRNA-derived stress-induced RNAs (mt-tiRNAs) in spermatozoa and if that is related to sperm quality.

Materials and Methods: Epididymosomes were isolated through precipitation from the epididymal fluid of C57BL mice fed ad libitum to a standard diet ($N = 3$) or HFD ($N = 4$) for 14 weeks. Epididymosome size distribution was assessed by nanoparticle tracking analyses. Epididymosomes and spermatozoa's mt-tiRNAs expression was assessed by quantitative polymerase chain reaction.

Results: No differences were found regarding epididymosome sizes between the control (1.26×10^{10} particles/epididymis) and HFD (2.21×10^{10} particles/epididymis) groups. Nonetheless, the HFD group presented an

enrichment in big epididymosomes suggesting a preference for microvesicle formation by outbudding of the plasma membrane though more studies are needed to test this hypothesis. We detected an increase in 3′mt-tiRNA-Met expression ($p = 0.0349$) in epididymosomes from the HFD group in comparison to the control diet. High levels of tiRNAs are correlated to the inhibition of protein synthesis. Our results support that hypothesis since spermatozoa after HFD presented a decreased mitochondrial 12S rRNA expression. Meanwhile, 3′mt-tiRNA-Ser expression was also decreased in spermatozoa after HFD ($p = 0.0050$), which could suggest a compensatory mechanism towards the epididymosome stimuli. Furthermore, spermatozoa's 3′mt-tiRNA-Ser expression was positively correlated with sperm normal morphology ($p = 0.0422$). Concurrently, a negative correlation was found between mitochondrial 12S rRNA expression in spermatozoa and the percentage of tail defects ($p = 0.0194$).

Conclusions: These preliminary results suggested that mt-tiRNAs produced by the epididymis, carried by epididymosomes, and delivered to spermatozoa impact spermatozoa's mitochondrial function. Furthermore, impaired mitochondrial dysfunction negatively impacts sperm quality, which could be a molecular mechanism by which excess weight promotes infertility.

57ASM – 071 | HOXB13: A prostate cancer biomarker potentially associated with obesity-related inheritance?

M. Pereira[1,2,3,4]; S. Pereira[1,2,4,5,6]; L. Rato[7,8]; P. Oliveira[5]; M. Alves[1,6]

[1]Endocrine and Metabolic Research, Unit for Multidisciplinary Research in Biomedicine, School of Medicine and Biomedical Sciences, Portugal; [2]Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, Portugal; [3]Faculty of Sciences of the University of Porto, Portugal; [4]Laboratory of Physiology, Department of Imuno-Physiology and Pharmacology, School of Medicine and Biomedical Sciences, Portugal; [5]LAQV-REQUIMTE and Department of Chemistry, Campus Universitario de Santiago, University of Aveiro, Portugal; [6]Department of Pathology, Faculty of Medicine, University of Porto, Portugal; [7]CICS-UBI-Health Sciences Research Centre, University of Beira Interior, Portugal; [8]Health School, Polytechnic Institute of Guarda, Portugal

Introduction: Obesity promotes homeostasis dysregulation. This condition can be promoted by high-fat diets (HFD) consumption and induce (epi)genetic alterations transgenerationally inherited. Prostate Cancer (PCa) is the fifth leading cause of death among men. PCa biomarkers, like Homeobox B13 (HOXB13), have been explored for diagnosis. Evidence suggested that HOXB13 can be modulated by the Fat mass and Obesity (FTO) whose dysfunction can be associated with obesity transgenerational inheritance. We hypothesized that the inheritance of obesity-related factors, such as FTO, can trigger the development of PCa in the offspring of individuals with obesity, particularly through HOXB13.

Materials and Methods: A transgenerational Mus musculus model was established, with F0 male mice exposed to different diets ($N = 8$ per group): standard, HFD (carbohydrate: 35.7%, protein: 20.5%, and fat: 36.0%), and diet correction ([DC], 60 days HFD, plus 120 days standard diet), ad libitium for 200 days post wearing. Mices were mated with lean females to generate F1 and F2 and were fed with chow diet. HOXB13 and FTO expression on the prostate of F0, F1 and F2 mice was assessed by quantitative Polymerase Chain Reaction. Data analysis was performed using analysis of variance followed by a Turkey post-hoc test for multiple comparisons.

Results: Results showed a positive correlation between HOXB13 and FTO expression in F0 and F1. FTO and HOXB13 expression remained unaltered in HFD prostates and their progeny, in comparison to the control. Interestingly, FTO and HOXB13 expression was increased in the prostate of F0 DC group but decreased in F1 and no differences were on F2.

Conclusion: Fathers exposed to HFD during early life, along with their sons (but not grandsons), appear to be at higher risk of developing PCa. These results highlight that HFD during early life can induce metabolic/molecular alterations that cannot be reverted by diet correction, impacting more than one generation.

57ASM – 075 | Chromosomal analyses – How are their importance in reproductive medicine in era of molecular genetics?

E. Vlad Gorduza[1]; V. Martiniuc[2]; M. Gramescu[1]; S. Popa[1]; L. Caba[1]; R. Popescu[1]; I. Resmerita[1]; M. Panzaru[1]; L. Butnariu[1]; A. Florea[2]; C. Rusu[1]

[1]"Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania; [2]"Cuza Voda" Obstetrics and Gynaecology Hospital, Iasi, Romania

Background: Infertility affects more than 48,000,000 couples. It represented the impossibility of a couple to have a pregnancy after 12 months of regular sexual intercourses. It be generated by different causes: anatomic, hormonal, infectious, immunologic, genetic. The most important genetic etiologic factor is the chromosomal anomalies.

Material and Methods: We made a retrospective study on couples with infertility. During the last ten years, we applied chromosomal analyses to 1.422 couples with infertility. We excluded from study the patients with clinic diagnosis of Turner and Klinefelter syndromes. f.

Results: From 1422 chromosomal analyses we identified 200 individuals with chromosomal variants (14.06%). The chromosomal variants were divided in two categories: chromosomal anomalies (91 cases) and chromosomal polymorphisms (109 cases). The chromosomal anomalies identified were: inversions (9 cases – 4.5% of chromosomal variants) balanced reciprocal translocations (23 cases – 11.5%) insertions (6 cases – 3%) Robertsonian translocations (16 cases – 8%) gonosomal mosaicism (31 cases – 15.5%) supplementary chromosomal material with unknown origin (add) (3 cases – 1.5%) unbalanced structural chromosomal anomalies (3 cases – 1.5%). The following chromosomal polymorphisms were identified: inversion of pericentric heterochromatin of chromosomes 1, 9 or 16 (38 cases – 19% of chromosomal variants) secondary constriction on chromosomes 1, 9, 16 or Y (50 cases – 25%) other polymorphisms that implied pericentric heterochromatin (9 cases – 4.5%) polymorphisms of satellites on the acrocentric chromosomes (12 cases – 6%). All polymorphisms were considered without clinical significance on the reproductive function and we offered a genetic counselling in correlation with this aspect. The chromosomal anomalies identified were pathogenic and we counselled the couple to address to a clinic for artificial reproduction technology.

Conclusion: Our study confirms the high frequency of chromosomal anomalies in couples with infertility and the importance of karyotype in study of such pathology.

57ASM – 084 | And what about antidepressants: Do they affect human spermatozoa?

R. A. Santos[1,2]; A. P. Sousa[1,3]; T. Almeida-Santos[1,3,4]; J. Ramalho-Santos[1,5]; R. S. Tavares[1,5]

[1]University of Coimbra, CNC- Center for Neuroscience and Cell Biology, Coimbra, Portugal; [2]University of Coimbra, Institute of Interdisciplinary Research (IIIUC), Coimbra, Portugal; [3]Hospital and University Center of Coimbra (CHUC), Reproductive Medicine Unit, Coimbra, Portugal; [4]University of Coimbra, Faculty of Medicine, Coimbra, Portugal; [5]Department of Life Sciences, Faculty of Sciences and Technology of University of Coimbra, Coimbra, Portugal

Introduction: Depression is the leading cause of disability worldwide, affecting about 280 million people. Worryingly its prevalence has been increasing, especially among the young and people of reproductive age, which translates into an increase in antidepressant (AD) consumption. ADs can cause some degree of sexual dysfunction in both men and women, however, their role in male fertility has been scarcely studied. In Portugal – the OECD country ranked 2nd in AD consumption – fluoxetine and sertraline are the most prescribed.

Materials and Methods: To assess sertraline and fluoxetine effects on sperm function and determine their in vitro mechanisms of action, spermatozoa were exposed to different concentrations (100 nM-10 μM) up to 24 h at 37°C and 5%CO2. Motility, viability (eosin-exclusion test), mitochondrial membrane potential (MMP; JC1 dye) ($n = 11$–13), chromatin/DNA integrity (Diff-Quik staining; $n = 10$), acrosome status (PSA-FITC staining) and tyrosine phosphorylation, an hallmark of capacitation (immunocytochemistry; $n = 10$), were assessed. Doses were chosen given the values for blood and seminal fluid reported in previous studies. Untreated controls were also used.

Results: Without affecting viability nor MMP at low concentrations, fluoxetine decreased progressive motility upon contact ($p < 0.05$ and 0.01), an effect still observed upon 24 h ($p < 0.05$ and 0.001). Furthermore, decreased sperm capacitation and increased chromatin integrity were observed after 3 h of exposure to 1 μM fluoxetine ($p < 0.05$). MMP was only affected following 24 h, at 10 μM ($p < 0.05$), a supraphysiological concentration. The acrosome status remained unaltered at all time points ($p > 0.05$). In contrast, sertraline only started affecting progressive motility after 3 h of incubation and at 1 μM ($p < 0.05$). This concentration decreased acrosome integrity, suggesting premature acrosome reaction, and increased chromatin integrity immediately after exposure ($p < 0.05$). Neither viability nor MMP were altered by sertraline ($p > 0.05$).

Conclusions: The most consumed ADs may impair sperm function at doses found in vivo, possibly through different mechanisms of action, ultimately compromising human male fertility.

57ASM – 112 | No loss, no gain: Lack of cholesterol efflux and tyrosine phosphorylation in overweight and obese men

M. Alfaiate[1,2]; R. A. Santos[1,2]; A. P. Sousa[1,3]; T. Almeida-Santos[1,3,4]; J. Ramalho-Santos[1,5]; R. S. Tavares[1,5]

[1]University of Coimbra, CNC- Center for Neuroscience and Cell Biology, Coimbra, Portugal; [2]University of Coimbra, Institute of Interdisciplinary Research (IIIUC), Coimbra, Portugal; [3]Hospital and University Center of Coimbra (CHUC), Reproductive Medicine Unit, Coimbra, Portugal; [4]University of Coimbra, Faculty of Medicine, Coimbra, Portugal; [5]Department of Life Sciences, Faculty of Sciences and Technology of University of Coimbra, Coimbra, Portugal

Background: Excess weight, a risk factor for infertility, is a global epidemic that is increasing over the years affecting 39% of the adult male population. In the Reproductive Medicine Unit of Centro Hospitalar Universitário de Coimbra 62% of men undergoing treatments/seminal analysis present excess weight, more than expected considering the general population, which is unexplained by up-to-date reports.

Material and Methods: Sperm samples from 944 men were collected, together with filled medical, lifestyle and exposure questionnaires, and divided into 4 groups: normal weight (body mass index; BMI < 25) with proven fertility (NWw/PF; $n = 65$), NW without PF (NWw/outPF; $n = 284$), overweight (OW; $25 \leq BMI < 30$; $n = 419$) and obese (Ob; BMI≥30; $n = 176$). Sperm quality was assessed according to WHO guidelines. Sperm functional markers, such as viability (eosin-Y), DNA status (Diff-Quik staining), acrosome integrity (PSA-FITC), tyrosine phosphorylation (anti-phosphotyrosine antibody) and cholesterol levels (Filipin III), were also addressed.

Results: No differences were found between groups ($p \geq 0.05$). However, when analysing samples before and after in vitro capacitation within groups, a decrease in viability and DNA integrity was observed in the NWw/outPF, OW and Ob groups ($p < 0.05$). Furthermore, OW and Ob also showed a decay in motility ($p < 0.05$). Tyrosine phosphorylation, a marker of sperm capacitation, was observed to increase only in NW groups, paralleled with a decrease in acrosome integrity ($p < 0.05$). To further understand the deterioration of function and capacitation in OW and Ob, levels of cholesterol were measured, as its membrane efflux is preponderant during capacitation. Once more, only the NW groups had significant cholesterol efflux during capacitation ($p < 0.05$), more prominent in men with PF ($p < 0.01$).

Conclusions: Capacitation, a process which allows sperm to become competent and fertilize, is impaired in OW and Ob. The exact role of cholesterol during capacitation is unknown but may explain the overflow of excess weight men to reproductive clinics, along with tyrosine phosphorylation.

57ASM – 124 | Linking Metabolic Changes in Sperm to Reduced Motility in Men with Asthenozoospermia

B. Guerra-Carvalho[1,2,3,4,5]; D. F. Carrageta[1,2,3]; S. C. Pereira[1,2,3,4,5]; A. Barros[5,6,7]; R. A. Carvalho[8]; M. G. Alves[1,2,3]; P. Domingues[9]; P. F. Oliveira[4]

[1]Clinical and Experimental Endocrinology, UMIB – Unit for Multidisciplinary Research in Biomedicine, ICBAS – School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal; [2]Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, Porto, Portugal; [3]Laboratory of Physiology, Department of Imuno-Physiology & Pharmacology, ICBAS – School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal; [4]LAQV-REQUIMTE and Department of Chemistry, University of Aveiro, Aveiro, Portugal; [5]Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal; [6]Centre for Reproductive Genetics Professor Alberto Barros, Porto, Portugal; [7]i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal; [8]Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal; [9]Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Background: Infertility affects 15% of reproductive-age couples and the male factor contributes to 50% of those cases. Sperm motility is crucial for fertilization and achieving a successful pregnancy. Asthenozoospermia is a common cause of male infertility, and its molecular mechanisms are unclear. Sperm motility is linked to metabolism, but the sperm metabolome is not well-characterized. Herein, we analysed the metabolome of sperm and seminal fluid. Furthermore, we investigated the redox status of the seminal fluid samples to gain insights into the oxidative stress levels in the reproductive tract.

Materials and Methods: Human seminal samples from normozoospermic ($n = 44$) and asthenozoospermic men ($n = 22$) were obtained from men seeking fertility counselling. Semen parameters were analysed according to the WHO guidelines. Spermatozoa were separated from seminal fluid through centrifugation. Water soluble

metabolites were extracted from spermatozoa and analysed by LC- Seminal fluid antioxidant potential and reactive oxygen species (ROS) content were assessed by FRAP assay and CM-H_2DCFDA probe, respectively. Protein nitration and lipid peroxidation levels in the seminal fluid were evaluated through slot-blot. Seminal fluid metabolome was analysed by ^{1}H-N Data were analysed using unpaired t-test and principal component analysis.

Results: We were able to identify 397 metabolites in sperm and 24 metabolites in the seminal fluid. Sperm metabolome had a distinctive profile between samples from normozoospermic and asthenozoospermic men. The sperm lysophospholipid content was significantly higher in asthenozoospermic men, whereas sperm phosphatidylethanolamine levels were increased in normozoospermic men. No differences were observed regarding the metabolome and the oxidative stress markers of the seminal fluid between normozoospermic and asthenozoospermic men.

Conclusions: Sperm metabolic signature differs between asthenozoospermic and normozoospermic men, indicating impaired metabolism in asthenozoospermia. Our results suggest exposure to increased inflammation during spermatogenesis or sperm maturation, which result in altered membrane fluidity and consequently decreased sperm motility.

57ASM – 126 | Is there interaction between Leiden mutations and familial Mediterranean fever?

H. Sargsyan

Yerevan State Medical University, Arabkir JMC, Yerevan, Armenia

Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by periodic attacks of fever with peritonitis, pleuritis, arthritis. The treatment of FMF is life-long colchicine therapy despite their some side effects (leukopenia, thrombocytopenia, hemolytic anaemia, coagulation defects, etc.). Over the past few years, several reports have been published, linking subclinical inflammation with markers of hypercoagulation and thrombosis in FMF. Some research shows that the factor V Leiden mutation has originated and accumulated in central European Caucasians.

Purpose: The aim of our study is to follow up non amyloid FMF patients which have at the same time the Leiden mutation carriers heterozygous.

Material and Methods: The field of survey was based on the evaluation of ten FMF pregnant females (age 22–26) who underwent assessment for gynaecological and haematological services. All patients were attack-free under regular colchicine treatment and under the control in FMF National Children Center from early childhood. FMF patients diagnosed in accordance with Tel-Hashomer criteria. Investigation of Folate metabolism and genetic polimorfism predisposing of thrombosis and thrombophilia were performed in Haematological Center.

Results: All patients from the haematologist assigned some medicines (Trombo ACC, Acidi folici, clexane, fraxiparine etc.) to regulate homeostasis and at the same time they were continuing Colchicine treatment.

Conclusion: Our started small survey indicates that assigning the above medications should be consider the hematologic side effects of colchicine to avoid negative outcomes of these medications and colchicine. In addition, it is so important to consider the contraindications of these drugs. Our short survey indicates that co-existence of FMF with Leiden mutations enables us to think about the relation between FMF and coagulation processes. It's feasible that FMF is a possible cofactor for thrombosis and thrombophilia. We will investigate coagulation factors with FMF pregnant females in our further research.

57ASM – 147 | Novel diagnostic markers for assessment of sperm damage in patients with testicular germ cell tumour

V. Pratap Sur[1]; O. Simoník[1]; E. Vacurova[2]; Z. Kratka[3]; R. Kren[4]; M. U. Pavel Skrobanek[5]; P. Postlerova[1]; J. Neuzil[2,6]; T. Hradec[7]; L. Zamecnik[7]; L. Boublikova[5]; T. Buchler[5,8]; K. Komrskova[1,9]

[1]Laboratory of Reproductive Biology, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic; [2]Laboratory of Molecular Therapy, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic; [3]Laboratory of Immunology, IVF Clinic GENNET, Prague, Czech Republic; [4]Laboratory of Embryology, IVF Clinic GENNET, Prague, Czech Republic; [5]Department of Oncology, First Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic; [6]School of Pharmacy and Medical Science, Griffith University, Southport, QLD, Australia; [7]Department of Urology, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic; [8]Department of Oncology, Second Faculty of Medicine and Motol University Hospital, Prague, Czech Republic; [9]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Introduction: The objective of this study is to investigate reproductive parameters of sperm and testicular germ cell pathologies associated with testicular germ cell tumour

(TGCT). The aim was to develop a novel diagnostic strategy to assess sperm quality before and after surgical and therapeutic interventions in TGCT patients. The outcome of the study is to select the optimal cryopreserved semen sample for assisted reproduction, based on a new set of diagnostic parameters.

Methods: Assessment of proliferation (Ki67 and H&E staining), sperm motility (CASA), acrosome stability (PNA), DNA integrity (TUNEL), mitochondrial activity (Oxygraph 2 k O2k, Seahorse XFp flux analyser, two-photon FLIM), gene regulation (qPCR/proteomic profiling), epigenetic modifications (H3K9ac, H3K36me3, H3K27me3), sperm ultrastructure (Cryo-SEM/Cryo-TEM).

Results: Assessment of sperm parameters and Ki67 marker evaluation determined impaired sperm function and tumour severity. Mitochondrial activity and oxygen consumption differed between TGCT and non-tumour tissue. Proteomic and genomic profiling revealed the downregulation of important genes (DAZL, DT1, GATA4, SUV39H1, EHMT1, EHMT2) and alterations in their protein expression. The epigenetic profile of sperm was significantly altered, requiring a comprehensive approach to understand these changes.

Conclusions: The study provided a comprehensive evaluation of TGCT-associated reproductive parameters from various experimental and clinical perspectives. Proteomic changes offer insights into TGCT development and differences in histone modification reflected a progression of testicular cancer and may impact early embryo development. A combination of CASA analysis for sperm motility with an assessment of acrosome damage correlated with DNA fragmentation is essential to predict the optimal IVF method. The damage to sperm structure, impacting fertility in TGCT patients was confirmed by ultrastructural analysis. Altered mitochondrial activity in the TGCT sperm and testicular tissue provided potential mitochondrial markers as diagnostic tools to assess sperm reproductive parameters.

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57ASM – 169 | Analysis of human sperm energy metabolism by Seahorse Extracellular Flux Analyser

O. Simonik[1]; V. Pratap Sur[1]; D. Spevakova[1]; P. Postlerova[1]; K. Komrskova[1,2]

[1]Laboratory of Reproductive Biology, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic; [2]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Introduction: In mammalian sperm, synthesis of ATP takes place via two main metabolic pathways, such as oxidative phosphorylation (OXPHOS) and glycolysis. The study of energetic status of spermatozoa is highly important since ATP is used for successful spermatozoa delivery to the site of fertilization. Extracellular flux analyser (Seahorse, Agilent®) represents excellent tool for detection of cell energy metabolism which can unveil subtle changes in sperm function related to patients' subfertility. The field of Andrology has small number of studies on sperm metabolism analysis often with inadequate experimental design. Aim of our study was to establish modified and reproducible protocol for human sperm energy metabolism analysis. We tested the effect of selected handling media and implied data normalization to increase precision and biological value of results.

Methods: Different media were tested: XF (Agilent®), modified Tyrode's solution (mSpTALP), modified human tubal fluid (mHTF). Spermatozoa (0.8×106 /well) were seeded into Concanavalin A-coated 8-well plates. Analysis was performed by Seahorse XFp with modified MitoStress and Glycolytic rate protocols. Sperm viability and mitochondrial membrane potential for data normalization were analysed by flow cytometry.

Results: Tested media had significant effect on sperm quality prior to analysis by Seahorse XFp analyser. Further, the results of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) representing level of OXPHOS and glycolysis, were also influenced by the media selection. Importantly, sperm viability and mitochondrial membrane potential were crucial for data normalization.

Conclusion: We report unique suitability of SpTALP media for human sperm energetic metabolism analysis by Seahorse XFp analyser and importance of mitochondria integrity analysis for data normalization. We have optimized and set up reproducible methodology for reliable monitoring of human sperm energy metabolism using Seahorse XFp analyser and validated this system for further clinical applications.

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57ASM – 217 | APC/C activity during transition from meiosis into mitosis

A. Horakova; L. Radonova; M. Konecna; M. Anger
Veterinary Research Institure, Brno, Czech Republic

Introduction: Early development in mammals is characterized by dramatic events, such as two consecutive meiotic divisions, fertilization and early cleavage cycles of blastomeres in the newly formed embryo. Moreover, during meiosis and the first embryonic cleavage cycles, cells remain transcriptionally silent, which renders regulated translation, posttranslational modifications and proteolysis essential for controlling cell cycle and chromosome segregation. During this period, oocytes, as well as blastomeres of early embryos, frequently suffer from aneuploidy. In fact, aneuploidy is the leading cause of failure of early development in mammals, including human. It is therefore vital to obtain better understanding of the cell cycle regulatory mechanisms during this period. In our study, we focused on the maintenance of essential cell cycle regulatory proteins, such as cyclins and securin, during transition from meiosis into mitosis, when the cells are transcriptionally silent.
Material and Methods: For our study, we used microinjection of NAs and confocal live cell imaging to quantitatively measure the expression of proteins in oocytes and embryos during early development. We also employed inhibitors to study the activity of enzymatic complexes, such as Anaphase Promoting Complex/Cyclosome (APC/C).
Results: Our results showed that the levels of important cell cycle regulators, such as securin and cyclins, is largely controlled by APC/C. This ubiquitin ligase is involved not only in their targeting during metaphase to anaphase transition, but also in maintaining their expression levels before division.
Conclusions and Recommendations: It seems that cells with silenced transcription, employ additional mechanisms to control protein expression of important cell cycle regulators. It remains to be, however, tested, whether this affects the frequency of aneuploidy during this period.
Funding: This work was supported by the Czech Science Foundation project 20-25850S and Grant RO0518 of the Ministry of Agriculture of the Czech Republic.

SYMPOSIUM 7 – EMBRYONAL PATHOLOGY

57ASM – 172 | Pathogenicity of fetal haemodynamic changes in development of congenital heart defects

D. Sedmera [1,2]
[1]*First Faculty of Medicine, Charles University, Prague, Czech Republic;* [2]*Institute of Physiology CAS, Prague, Czech Republic*

Unlike most embryonic and fetal organs, the heart function is essential for continued survival of the developing organism. The heart moulds itself into its final form through genetic program as well as in response to functional demands, transmitted via hemodynamic cues. Thus, changes in hemodynamics reflect on heart form, and experimental perturbations were shown to result in reproducible heart malformations.
I will discuss the use of the chick embryo as a prime experimental system to studying links between hemodynamics and development of heart malformations. Hemodynamic perturbations can be induced either mechanically by clipping/ligating inflow or outflow vessels, or via pharmacological interventions that manipulate the preload or afterload or influence the heart rate. I will summarize the most significant findings obtained in this model system, mention also alternative models such as developing zebrafish or mammalian fetus, and provide translation to potential human situations that occur in clinical practice.

57ASM – 189 | A novel role for NADPH oxidases in fetal programming of pulmonary hypertension

A. Görlach [1,2]
[1]*Experimental and Molecular Paediatric Cardiology, German Heart Center Munich, Munich, Germany;* [2]*School of Medicine, Technical University of Munich, Munich, Germany*

Increasing evidence indicates that adverse circumstances during pregnancy can result in permanent changes to fetal physiology and have life-long implications for offspring health. Fetal hypoxia is a common consequence of complicated pregnancies including preeclampsia, maternal obesity, gestational diabetes, and inflammatory diseases. Increasing evidence suggests that dependent on time and severity fetal hypoxia might enhance the risk to develop chronic diseases later on in life. While epigenetic mechanisms are suggested to contribute to fetal programming of disease indepth insights into the underlying mechanisms are scarce.

As we have previously shown that reactive oxygen species generated by the enzyme family of NADPH oxidases contribute to the response to hypoxia in adulthood we hypothetized that these enzymes might also interfere with the fetal response towards hypoxia and programming of disease in adulthood.

57ASM – 219 | Hypoxia signalling and cardiac development: From metabolic regulation to congenital cardiac defects

S. Mendoza-Tamajon[1,2]; S. Urra[1]; B. Escobar[2]; T. Albendea-Gomez[1,2,3]; C. Diaz-Diaz[2],. S. Martin-Puig[1]
[1]Biomedical Research Institute Alberto Sols, CSIC-UAM, Madrid, Spain; [2]National Center for Cardiovascular Research, CNIC, Madrid, Spain; [3]Francisco de Vitoria University, UFV, Madrid, Spain

Introduction: Congenital Heart Defects (CHDs) are the most common structural abnormalities affecting 1% of all human births. The molecular foundations of these pathologies remain unclear. Hypoxia represents a well-recognized environmental factor increasing the risk of CHDs, while the mechanisms connecting HIF and cardiac defects are indeterminate.
Aim: to understand the importance of VHL/HIF signalling in cardiovascular progenitors (CVPs) contributing to different cardiac cell types and structures.
Methods: we generated novel conditional mutants of gain and loss of function or double knock out mice of the hypoxia pathway in first and second heart field CVPs and determined the impact of altering VHL/HIF signalling during cardiogenesis by performing haematoxylin/eosin staining, immunohistochemistry, echocardiography, western blot, RNAseq or q-PCR on these models.
Results: we defined the spatiotemporal pattern of HIF1α with enriched expression on the compact myocardium and a progressive reduction between E9.5-E14.5. We uncovered that HIF1α controls cardiac metabolic compartmentalization with a glycolytic mark on the compact myocardium and a mitochondrial-oxidative program on the trabecula that is essential to allow cardiomyocyte maturation, expression of conduction system genes, ventricular chamber formation and cardiac function (1). However, elimination of HIF1α does not preclude cardiac development and leads to HIF2α and ATF4 induction, favouring a transient amino acid catabolic program to compensate the lack of glycolysis in the absence of HIF1α (2). HIF2α is expressed in the ventricular endocardium by midgestation and its ectopic overexpression on Nkx2.5 CVPs upon VHL/HIF1 double inactivation leads to interventricular septal (IVS) defects and semilunar valve hypertrophy. Elimination of VHL in second heart field CVPs results in right ventricular thinning, impaired trabeculation and IVS malformation, causing embryonic lethality.
Conclusions: our data demonstrate that HIF signalling is dynamically and locally modulated in different pools of CVPs, governing metabolism and other processes important for the correct formation of ventricular chambers, IVS and valves.

References:

1. Menendez-Montes I, Escobar B, Palacios B, Gómez MJ, Izquierdo-Garcia JL, Flores L, Jiménez-Borreguero LJ, Aragones J, Ruiz-Cabello J, Torres M, Martin-Puig S. Myocardial VHL-HIF Signaling Controls an Embryonic Metabolic Switch Essential for Cardiac Maturation. Dev Cell. 2016 Dec 19;39(6):724–739. doi: 10.1016/j.devcel.2016.11.012
2. Menendez-Montes I, Escobar B, Gomez MJ, Albendea-Gomez T, Palacios B, Bonzon-Kulichenko E, Izquierdo-Garcia JL, Alonso AV, Ferrarini A, Jimenez-Borreguero LJ, Ruiz-Cabello J, Vázquez J, Martin-Puig S. Activation of amino acid metabolic program in cardiac HIF1-alpha-deficient mice. iScience. 2021 Feb 3;24(2):102124. doi: 10.1016/j.isci.2021.102124

57ASM – 233 | A new perspective on embryonic metabolism in diabetic pregnancies

C. Kappen[1]; K. P. Stone[1]; C. Kruger[1]; Q. Chen[2,3]; S. S. Gross[2,3]; J. M. Salbaum[4]
[1]Department of Developmental Biology; [4]Laboratory of Regulation of Gene Expression; [2]Pennington Biomedical Research Center/Louisiana State University System, Baton Rouge, Louisiana, USA; [3]Department of Pharmacology, Weill Medical College of Cornell University, New York City, New York, USA

Diabetes during pregnancy is a known teratogen, raising the risk for structural birth defects, such as heart malformations and defective neural tube closure. The generally accepted view is that the culprit is the excess blood glucose from the mother, because better glycemic control in diabetic women is associated with fewer adverse pregnancy outcomes and lower birth defect incidence. Animals with experimental hyperglycemia, repeated high-dose administration of glucose to pregnant rodents and morphogenetic defects in rodent embryos cultured in the presence of high glucose levels, all provide evidence for linking excess glucose to birth defects. On the other hand, the majority of human pregnancies affected by maternal diabetes do yield offspring without birth defects, and incomplete penetrance of anomalies in diabetic rodent pregnancies also

raises questions whether additional factors may contribute to birth defect risk in these pregnancies. Some authors have implicated, for examples, maternal hyperlipidemia and high ketone levels, that –in combination with maternal hyperglycemia– create conditions of nutrient overload, leading to so-called "fuel-mediated teratogenesis". Contrary to this prevailing dogma, work from our laboratories with mouse models provides multiple lines of evidence that birth defects in diabetic pregnancies are caused by nutrient shortage, in early developmental stages before a functional placenta is formed. Unbiased transcriptomic profiling reveals that embryos in diabetic conditions experience nutritional stress, detectable at the level of individual embryonic cells. Remarkably, a single bolus of glucose can alleviate the adverse exposure, indicating a deficient supply of glucose to the embryo. Concomitantly, we observe nutrient retention in the visceral yolk sac, which is the major source of nutrients before the embryonic circulatory system is connected to the placenta. Further support for our nutrient deficit hypothesis comes from untargeted metabolomics approaches, as well as well-documented developmental delay and intrauterine growth reduction in rodent diabetic pregnancies. We posit that the re-evaluation of prior findings required in light of our results will lead to a major paradigm change in the field. Potential implications for the care of women with diabetes, and strategies for prevention of birth defects in their offspring, will be discussed.

SYMPOSIUM 8 – PERSONALIZED AND GENDER MEDICINE

57ASM – 078 | Sex-specific differences in myocardial glucose metabolic rate in non-diabetic, pre-diabetic and type 2 diabetic subjects

V. Cassano[1]; G. Armentaro[1]; F. Giofré[1]; P. Vizza[1]; P. Veltri[2]; G. L. Cascini[3]; G. Sesti[4]; A. Sciacqua[1]; E. Succurro[1]

[1]University Magna Graecia, Department of Medical and Surgical Science, Catanzaro, Italy; [2]University of Calabria, Department of Computer, Modelling, Electronic and Systems Engineering, Cosenza, Italy; [3]University Magna Graecia, Department of Experimental and Clinical Medicine, Catanzaro, Italy; [4]University Sapienza, Department of Clinical and Molecular Medicine, Roma, Italy

Introduction: Type 2 diabetes (T2DM) women have higher risk for cardiovascular disease (CVD) than men. In both pre-diabetic and T2DM subjects was observed a myocardial insulin resistance, considered a causal factor in development of CVD. However, it is not yet defined whether exist sex-related differences in myocardial insulin resistance in diabetic and pre-diabetic subjects. We evaluated sex-related differences in myocardial glucose metabolic rate (Glu) in normal glucose tolerant (NGT), pre-diabetic and T2DM subjects.

Methods: We examined 57 subjects with NGT, pre-diabetes, T2DM. Myocardial Glu was evaluated using dynamic cardiac 18F-FDG PET combined with euglycemic hyperinsulinemic clamp, which allows to assess peripheral insulin sensitivity normalized for lean body mass (MFFM) and to standardize metabolic and hormonal conditions during PET. The 18F-FDG PET imaging procedure started 60 min after the insulin infusion. The insulin-glucose infusion continued throughout the PET imaging sequence, maintaining euglycemia by continuous adjustment of the glucose infusion rate according to glucose levels of the arterialized blood samples collected every 5 min.

Results: Pre-diabetic and T2DM women exhibited greater relative differences in whole-body insulin-stimulated glucose disposal and myocardial Glu than pre-diabetic and T2DM men, compared to NGT. Compared to NGT women, pre-diabetic women exhibited 35% decrease in myocardial Glu ($p = 0.01$) and those with T2DM 75% decrease ($p = 0.005$). Compared to NGT men, only T2DM men exhibited 41% reduction in myocardial Glu ($p = 0.007$), while no significant difference was observed with pre-diabetic men. The statistical test for interaction between sex and glucose tolerance on myocardial Glu ($p < 0.0001$) was significant suggesting a sex-specific association.

Conclusions: An impairment in myocardial glucose metabolic rate is an early alteration already observed in pre-diabetic women at risk for T2DM. The sex-specific myocardial insulin resistance could be an important key factor responsible for the greater effect of T2DM on the excess risk of CVD in women than in men.

57ASM – 080 | Short term effect of Sodium-Glucose-Cotransporter-2-Inhibitors and Sacubitril/Valsartan on comprehensive geriatric assessment and oxidative stress in elderly with chronic-HF (from MAGIC-HF)

G. Armentaro[1]; M. Magurno[1]; V. Cassano[1]; C. A. Pastura[1]; E. Clausi[1]; V. Condoleo[1]; S. Miceli[1]; E. Succurro[1]; F. Arturi[1]; G. Sesti[2]; A. Sciacqua[1]

[1]Mater Domini University Hospital of Catanzaro – Geriatric Department, Catanzaro, Italy; [2]Sapienza University of Rome, Rome, Italy, Rome, Italy

Introduction and Background: Sodium-Glucose-Cotransporter-2-inhibitors (SGLT2i) have improved

clinical prognosis in patients affected by heart failure (HF) with reduced ejection fraction (HFrEF). Cognitive impairment, depression, and poor physical functional performance are frequently in patients with HF and result in a worse prognosis. The aim of this study was to evaluate the effects of SGLT2i on functional, humoral, and cognitive aspects, assessed by performing a comprehensive geriatric assessment (CGA), and on oxidative stress and platelets activation biomarkers, in a cohort of HFrEF elderly, and any differences between men and women.

Materials and Methods: We enrolled 91 HFrEF patients (63 men and 28 women, mean age 73.7 ± 4.7 years). SGLT2i therapy was introduced in patients already treated with Sac/Val for at least 12 months.

Results: After 3 months follow-up, we observed a significant improvement in cognitive, humoral and functional parameters of CGA, NTpro-BNP levels and echocardiographic parameters. Changes (Δ) in Montreal Cognitive Assessment (MoCA) ($p = 0.015$) and Cardiac Index (CI) which were greater in men ($p < 0.0001$) and Δ in Geriatric Depression Scale (GDS) which were greater in women ($p = 0.029$). In the whole population, multivariate analysis shows that Δ of CI, Homeostatic model assessment (HOMA), Sp-Selectina, Nox-2 and 8-Isoprostane contributed for 19.7%, 9.4%, 6.4%, 3.8% and 2.9% to Mini mental state examination (MMSE) variability, respectively, for a 42.2% of MMSE variation; moreover Δ of HOMA, Sp-Selectina and hs-CRP contributed for 21.6%, 5.7% and 4.0% to MoCA variability, for a 33.3% of MoCA variation. In addition, Δ of Sp-Selectina, Nox-2, CI and HOMA globally contributed for 37.5% of GDS variation ($p < 0.05$); and Δ of HOMA and Sp-Selectina contributed for 30.9% of Short performance physical battery variation ($p < 0.0001$).

Conclusions and Recommendations: This study shows that echocardiographic and cognitive improvements induced by SGLT2i are greater in men than in women, instead metabolic and humoral improvements are greater in women.

57ASM – 119 | Gender-based effect of Ramadan fasting on anthropometric markers, fat deposition, and metabolic profile

J. S. JohnBritto[1]; M. Khalil[1]; H. Abdallah[1]; I. Farella[1]; E. Lanza[1]; S. Santoro[1,2]; A. Di Ciaula[1]; L. Bonfrate[1]; G. Garruti[3]; P. Portincasa[1]

[1]Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy; [2]R&D resp. – Eurisko Technology Srl, Modugno, Italy; [3]Section of Internal Medicine, Endocrinology, Andrology, and Metabolic Diseases, Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari Medical School, Bari, Italy

Background: Intermittent fasting has been linked with several beneficial metabolic effects, including weight loss. Ramadan fasting is a model of intermittent fasting followed by millions of Muslims during the whole lunar month of the year. The existence of possible gender-based differences in anthropometric indices during Ramadan fasting is still unexplored.

Methods: Anthropometric indices, subcutaneous/visceral fats (ultrasound at 5 different sites using a Sonoscape E2 Exp by Fujifilm Ultrasound machine provided by Eurisko Technology srl and equipped with 1–7 Mhz 3C-A convex probe set at 3.5 Mhz), glucose and lipid profiles were measured before and after Ramadan fasting period (29 days) in 21 Muslim subjects (age: 30.6 ± 1.4 years; female 52.4%, body mass index: 23.3 ± 0.7 kg/m2) from the Mediterranean basin living in Italy. The physical activity and dietary habits were assessed by semi-qualitative questionnaires before, during, and after the Ramadan period.

Results: Ramadan induced in both genders a significant ($p < 0.05$) reduction in body mass index (males −4.2%, females −3.8%) and waist circumference (males −6.0%, females −6.6%). Ramadan in females was associated with decreased subcutaneous and abdominal visceral fat. Ramadan in males was associated with a reduction of glycemia (from 92.7 ± 9.4 to 86 ± 11.6 mg/dL, at baseline and after, $p = 0.02$), insulin serum levels (from 7.2 ± 3.7 to 5.8 ± 3.4 uM/mL, $p = 0.01$), and insulin resistance (HOMA index, from 1.7 ± 0.3 to 1.3 ± 0.2, $p = 0.01$). Caloric intake and physical activity did not change in both genders.

Conclusion: Ramadan intermittent fasting has effects on both genders in terms of weight loss and improved metabolic homeostasis, irrespective of caloric intake and physical activity. Significant gender-based metabolic differences emerge from this study, and the role of the gut microbiome is being investigated.

57ASM – 120 | Gender-based differences in the response to biological therapies for severe asthma

C. Pelaia[1]; A. Casarella[1]; G. Pelaia[1]; G. Marcianò[1]; V. Rania[1]; L. Muraca[1]; E. Cione[1]; L. Bianco[1]; C. Palleria[1]; B. D'Agostino[2]; D. Mazzuca[1]; G. De Sarro[1]; G. Di Mizio[1]; A. Sciacqua[1]; L. Gallelli[1]

[1]University Magna Graecia, Catanzaro, Italy; [2]University of Campania Luigi Vanvitelli, Naples, Italy

Introduction: Biological therapies are used to treat severe asthma inducing an improvement of clinical symptoms and lung function. Data on gender difference of these drugs in patients with severe asthma are lacking.

Material and Methods: The present study aimed to evaluate the effects of sex-related differences on biological drugs in patients with severe asthma. In this observational, open-label, prospective, non-controlled, single-center cohort pilot study, we recruited adult patients aged >18 years diagnosed with severe asthma and not previously treated with biological drugs. The first clinical end point was the statistical difference ($p < 0.05$) in the efficacy of biological drugs assessed using the asthma control test and lung function between sexes. The first safety end point was the statistical difference ($p < 0.05$) in developing adverse drug reactions between gender.

Results: We recruited 74 patients with severe asthma (48 women and 26 men) with a mean age of 59.4 (standard deviation, 11.8) years. The mean forced expiratory volume in one second was 1.69 (standard deviation, 1.39) L for women and 1.94 (standard deviation, 1.07) L for men, and increased significantly after the treatment ($p < 0.01$), with no significant differences in gender ($p = 0.8$). Moreover, asthma control test improved one year after the beginning of the biological therapy without significant differences between men and women ($p = 0.5$). The most common prescribed drug was omalizumab (45.9% of the patients; $p < 0.01$) without significant differences between gender ($p > 0.05$). We did not detect adverse drug reactions during the observation period.

Conclusions and Recommendations: In conclusion, in asthmatic patients, gender does not have an impact in either the effectiveness or safety of biological therapies used to treat severe asthma.

57ASM – 134 | Sex-linked cardiovascular risk in patients with metabolic-associated fatty liver disease (MAFLD)

A. Montesano; E. Lanza; A. Di Ciaula; M. Khalil; I. Galerati; H. Abdallah; A. Belfiore; L. Bonfrate; P. Portincasa

Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy

Introduction: The acronym MAFLD (Metabolic Associated Fatty Liver Disease) has been proposed to underline the coexistence of fatty liver and metabolic alterations, with relevant implications in terms of cardiovascular disease (CVD) risk. In this context, a sex effect is possible. We therefore calculated the CVD risk in MAFLD patients divided according to sex.

Methods: A total of 63 patients (24 females) who met the inclusion criteria for MAFLD underwent assessment of anthropometric indices and fatty liver grade by ultrasound, serum glucose, liver enzymes and lipid profiles. The CVD risk was estimated by the ASCVD (atherosclerotic cardiovascular disease) risk calculator (American College of Cardiology), which determines the 10-year risk of heart disease or stroke.

Results: Overall, the Body Mass Index was comparable between sexes (F:30.1 ± 1.1Kg/m2, M:30.4 ± 1.4 Kg/m2, p = NS), as also serum glucose, lipid profile, and liver enzymes, except than HDL-cholesterol level, which was higher in females (56.1 ± 3.7 mg/dL) than in males (41.6 ± 4.8 mg/dL, $p = 0.02$). Females presented a higher prevalence of mild steatosis (59.1%) than males (31.4%, $p = 0.04$), while the prevalence of moderate and severe steatosis was comparable across sexes. The CVD risk was more than double in males (19.2%), than in females (6.9%, $p = 0.02$), and increased with the grade of fatty liver only in males (6.7%, 23.2%, and 33.3% in mild, moderate, and severe steatosis respectively).

Conclusion: The link between MAFLD and CVD seems more evident in males than in females, irrespective of Body Mass Index, serum glucose and lipid profile. In males, but not in females, the increase in CVD risk seems proportional to the severity of fatty liver. Further studies are needed to better explore the role of gender in pathogenic pathways linking MAFLD and CVD risk.

57ASM – 196 | Unique kinase inhibitor targeting specifically MLL/FLT3-mediated signalling for personalized medicine in AML

N. Niederlova[1]; P. Koralkova[1]; P. Chaloupkova[1]; I. Hradilova[1]; J. Gursky[1]; B. Cudova[2]; H. Urbankova[2]; E. Reznickova[3]; T. Gucky[3]; D. Myslivcova[4]; M. Belickova[4]; V. Krystof[3]; V. Divoky[1]
[1]*Department of Biology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic;* [2]*Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacký University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic;* [3]*Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic;* [4]*Institute of Haematology and Blood Transfusion, Prague, Czech Republic*

Background: Mutations in Fms-like tyrosine kinase 3 (FLT3) gene represent the most frequent alterations in acute myeloid leukaemia (AML). Overexpression of FLT3, Src family kinases (SFK) and salt-inducible kinases (SIK) has been demonstrated in mixed lineage leukaemia-rearranged (MLL-r) leukaemias. Specific targeting of MLL-r/FLT3 represents a great challenge in haematology. We have developed a potent kinase inhibitor LGR3922, that exhibits nanomolar potency against FLT3-ITD, SFK, MAP kinase-interacting serine/threonine-protein kinase 2 (MNK2) and Ca2+/calmodulin-dependent protein kinase II (CaMKII) in biochemical/cellular assays. In MLL-r xenografts, LGR3922 shows rapid response to treatment, superior efficacy in tumour prevention and regression. LGR3922 also effectively inhibits colony formation of primary AML progenitors and specifically eliminates FLT3-ITD and KMT2A::MLLT3 mutant cells. The high efficacy of LGR3922 towards MLL-r cells led us to hypothesize that other targets, SIK1/2 and/or CDC2-like kinase 1 (CLK1) identified in the kinase assays, may be involved in the inhibitory activity.
Aim: Additional characterization of compound LGR3922 and evaluation of the contribution of SIK1/2 and CLK1 in inhibition on MLL-r/FLT3-mediated signalling in AML cells.
Methods: Antiproliferative effect of LGR3922 was tested on MLL-r cell lines using the resazurin assay. Inhibitory activity of LGR3922 on selected targets of MLL-r/FLT3-mediated signalling was evaluated by immunoblotting or real-time PCR.
Results: A dose-dependent inhibition of SIK targets, histone deacetylase 4 (HDAC4) and/or MEF2C was observed in LGR3922-treated MV4-11 and MOLM-13 cells. LGR3922 also showed potent inhibitory activity against serine/arginine-rich (SR) proteins (targets of CLK1) in these cells.
Conclusions: LGR3922 specifically inhibits several AML kinases: FLT3-ITD, SFK, CaMKII, MNK2, SIKs and CLK1 in vitro and/or in vivo. Simultaneous inhibition of these targets effectively blocks MLL-r/FLT3-mediated signalling resulting in proliferation arrest of MLL-r/FLT3-positive cells. LGR3922 is a unique candidate molecule for personalized AML therapy.
Funding: Program EXCELES, ID Project No. LX22NPO5102; GAČR 23-05462S, UP Young Researcher Grant Competition, ID Project JG_2023_016.

57ASM – 199 | Altered hypoxic response in Alzheimer's disease patient-derived fibroblasts: What (H)IF gender matters?

S. Correia[1,2,3]; P. I. Moreira[1,2,4]
[1]*CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal;* [2]*CIBB – Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal;* [3]*Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal;* [4]*Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

With the aging of the population, the burden of Alzheimer's disease (AD) is rapidly escalating to epidemic proportions. At the epicentre of AD epidemic are women. Epidemiological evidence reported a higher incidence and severity of AD in women. In this sense, to demystify the molecular basis behind this sex/gender predisposition and vulnerability to AD may constitute an important step for AD patient stratification and precision medicine. Considering that the early stages of AD are marked by defects in brain oxygenation, this study is devoted to unveil the sex/gender-specific response to severe hypoxia (1% O_2) in AD using AD patient-derived fibroblasts and correspondent fibroblasts from sex- and age-matched healthy individuals. Preliminary results revealed an opposite impact of severe hypoxia on the levels of the master regulator of oxygen homeostasis, as denoted by a significant increase in HIF-1α levels in male AD patient-derived fibroblasts, while a drastic reduction was observed in female AD patient-derived fibroblasts when compared with the respective controls. Interestingly, a marked reduction in HIF-2α levels was found in both male and female AD patient-derived fibroblasts when compared with the respective controls. Additionally, a reduction in PHD2 levels, a negative regulator of HIF-α, was also detected in both male and female AD patient-derived fibroblasts under normoxic and hypoxic conditions. As expected, these effects

in hypoxic signalling were accompanied by changes in mitochondrial function and dynamics, being the deleterious impact of severe hypoxia more pronounced in female AD patient-derived fibroblasts. Overall, this preliminary data suggests a faulty response to severe hypoxia in female AD-derived fibroblasts, which in part may explain the sex/gender differences in AD and open "gateways" for new and personalized therapeutic interventions.

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57ASM – 210 | Subclinical atherosclerosis as a prognostic marker for hypertension

D. Abidova[1]; J. Uzokov[2]; M. Mukhamedova[3]
[1]*Republican specialized scientific practical medical center of Cardiology, Tashkent, Uzbekistan;* [2]*Republican specialized scientific practical medical center of therapy and medical rehabilitation, Tashkent, Uzbekistan;* [3]*Center for the development of professional qualification of medical workers, Tashkent, Uzbekistan*

Introduction: Subclinical atherosclerosis is a marker for development of future atherosclerotic cardiovascular disease. However, it is still unclear whether subclinical atherosclerosis predicts hypertension in patients who have not diagnosed it yet. Aim of the study was to evaluate possible significance of subclinical atherosclerosis for the development of hypertension in patients who have not diagnosed hypertension yet.

Materials and Methods: 418 middle aged adults who had subclinical atherosclerosis and without hypertension enrolled in this study (aged 31–49 years, mean age 39.12 ± 12.0 years, male = 53%). All participants were examined general clinical, anthropometric, laboratory and instrumental measures. Subclinical atherosclerosis were diagnosed using ultrasound methods via ankle-brachial index (ABI) using established cutoffs. All patients followed up to 6 years. Mean follow up was 4.5 ± 2.1 years. All statistical analysis were performed using STATA software.

Results: During the mean follow up period 17% (71) patients were diagnosed hypertension (SPB < 140 mmHg and/or DBP < 90 mmHg). Among them 12% (50) were males whilst 5% (21) were females and men tended to have more levels of blood pressure than women did ($p < 0.05$). During the follow up period, subclinical atherosclerosis level has increased more than 20% to those who was diagnosed hypertension. Patients who developed hypertension tended to have more smokers (OR 1.58, 1.14–2.28, CI 95%, $p < 0.05$), BMI < 30 kg/m2 (OR 1.57, 1.12–1.98, CI 95%, $p < 0.05$), family history of hypertension (OR 1.45, 1.09–1.87, CI 95%, $p < 0.05$), frequent stress at work (OR 1.35, 1.06–1.82, CI 95%, $p < 0.05$) and financial problems at home (OR 1.23, 1.05–1.72, CI 95%, $p < 0.05$).

Conclusions: Subclinical atherosclerosis is a predictor for new onset hypertension in men especially for those who were smoker, obese and had family history of hypertension as well as frequent stressed at work.

SYMPOSIUM 9 – ENDOCRINOLOGY

57ASM – 023 | Ubiquitination default as a familial non-medullary thyroid cancer marker

C. Fernandes[1,2,3,4]; E. Teixeira[1,2,5]; T. Gaspar[1,2,4,5]; S. Canberk[1,2,5]; R. Batista[1,2]; P. Soares[1,2,5]
[1]*I3s – Instituto De Investigação E Inovação em Saúde, Porto, Portugal;* [2]*Ipatimup – Instituto de Patologia E Imunologia Molecular da Universidade do Porto, Porto, Portugal;* [3]*FCUP – Faculdade de Ciências da Universidade do Porto, Porto, Portugal;* [4]*ICBAS – Instituto Ciências Biomédicas Abel Salazar, Porto, Portugal;* [5]*FMUP – Faculdade de Medicina da Universidade do Porto, Porto, Portugal*

Introduction: Hereditary thyroid cancer accounts for approximately 10% of all thyroid cancer cases. Familial non-medullary thyroid cancer (FNMTC) arises from thyroid follicular cells and is diagnosed when the disease is present in two or more first-degree relatives in the absence of other known familial syndromes. A 36 year-old woman was diagnosed with oncocytic variant of papillary thyroid carcinoma, along with her twin 19 year-old daughters. By Next Generation Sequencing (NGS) a germline mutation (p.G486R) in ubiquitin-specific protease 42 gene (USP42) was found. USP42 belongs to the family of deubiquitinating enzymes (DUBs), known for regulating cell cycle arrest, apoptosis, and other relevant cellular functions. Consistent evidence has shown that deregulation of DUBs may lead to tumorigenic processes.

Aim: Validate USP42 alteration as an underlying cause of FNMTC.

Materials and Methods: To validate our hypothesis we are currently conducting in silico analysis resorting to online NGS databases. In vitro assays using Nthy-ori 3–1 cell line are being performed. siRNAs are being used to silence the USP42 gene. CRISPR-RNP methodology will be used to knockout the USP42 gene and also to knock-in the p.G486R mutation found in this family. Morphologic and

functional assays will be performed in the transformed cell clones. Finally, in vivo assays will be conducted in zebrafish to replicate in vitro results.

Results: Through 2 NGS databases, we inferred that USP42 has an augment in copy number variation (CNV) and sporadic mutations can occur. Human thyroid tissue normally expresses the USP42 gene. By siRNAs we successfully silenced this gene in human thyroid Nthy-ori 3–1 cell line, as verified by RT-PCR RNA analysis. Protein expression is currently being analysed by Western blot technique and cellular functional assays are being conducted at the same time.

Conclusion: If our hypothesis is verified, USP42 may be used as a potential prognostic marker on FNMTC.

57ASM – 175 | Estrogens decrease NAD+ levels and Sirtuin3 activity in osteoclast precursor to maintain bone mass

A. Carvalho[1,2]; V. Sardão[3]; H.-N. Kim[4]; M. Almeida[4]
[1]Center For Neurosciences And Cell Biology, Cantanhede, Portugal; [2]PhD Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal; [3]Multidisciplinary Institute of Aging (MIA-Portugal), University of Coimbra, Coimbra, Portugal; [4]Division of Endocrinology and Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, University of Arkansas for Medical Sciences, Little Rock, USA

Osteoclasts are giant multinucleated cells responsible for bone resorption. Loss of estrogens at menopause increases osteoclasts and causes bone loss. RANKL is the critical cytokine for osteoclastogenesis and stimulates mitochondrial respiration in osteoclast precursors. 17β-estradiol (E2) signalling prevents this effect, stimulates apoptosis, and decreases osteoclastogenesis. During oxidative phosphorylation, NADH is oxidized to NAD+. When mitochondrial function is compromised, the ratio of NAD+/NADH decreases and the activity of NAD+-dependent enzymes such as the mitochondrial deacetylase Sirt3 can be reduced. Here, we examined the contribution of NAD+ and Sirt3 activity to the anti-osteoclastogenic effects of estrogens in vitro and in vivo.

Microarray analysis was performed in bone marrow macrophage (BMMs) without RANKL, and after 2 or 5 days with RANKL. Sirt3 activity and NAD+/NADH were measured with commercial assay kits. Osteoclast were enumerated after tartrate-resistant acid phosphatase staining. Degradation of DEVD-AFC was used to measure caspase-3. Mice with conditional deletion of Sirt3 or Nampt in the osteoclast lineage (LysM-Cre) were generated. DXA was used to evaluate bone mineral density (BMD).

Microarray analysis indicated that RANKL increased the expression of genes associated with NAD metabolism in osteoclast precursors. Addition of RANKL to BMMs for 6 h promoted an increase in NAD+ and NAD+/NADH ratio, along with Sirt3 activity, while E2 inhibited these effects. The NAD precursor NR increased NAD+ levels, NAD+/NADH ratio, Sirt3 activity, and attenuated the anti-osteoclastogenic effects of E2. Addition of FK866, a specific inhibitor of the NAD salvage pathway, decreased NAD+ levels, mitochondrial function and promoted cell death, similar to E2. The loss of BMD following ovariectomy for 6 weeks was attenuated in mice with deletion of NAMPT or Sirt3.

Our results suggest that the inhibitory actions of E2 on osteoclastogenesis and bone resorption are mediated by a decrease in NAD+ and Sirt3 activity.

57ASM – 218 | Generation of thyroid hormone receptors loss-of-function models to dissect the role of maternal thyroid hormone during zebrafish neurodevelopment

A. Andrade[1]; L. Rodrigues[2]; M. Campinho[1,3]
[1]Algarve Biomedical Center-Research Institute, Faro, Portugal; [2]Superior School of Health- University of Algarve, Faro, Portugal; [3]Faculty of Medicine and Biomedical Sciences of the University of Algarve, Faro, Portugal

Maternal thyroid hormone (T3) is essential during vertebrate neurodevelopment. T3 cellular signalling is highly regulated from its intracellular transport, deiodination and binding to its nuclear receptors. Thus, the action of the T3 is dependent on the cellular context, timeframe, and the target cell expression profile of T3-regulatory/effector genes. Pivotal to T3-action are thyroid receptors, thra and thrb. Therefore, to understand T3 action it is necessary to understand thyroid hormone receptor function. Then, were analysed the results in zebrafish offspring whose thraa and mct8 was deleted by CRISPR-Cas9, verifying the phenotype in HLof individuals. Furthermore, was evaluated the cytoarchitecture of the spinal cord throught WIHC in thraa mutants enhancing the neurons with antibody anti-HUC and glial cells with antibody anti-ZRC. In the mct8 mutants was performed WISH evaluating the expression of pax6a in progenitor neural cells and PH3 in mitotic cells. In addition, the genetic compensation was tested in the thraa knockout model performing the qPCR of thrab and thrb paralog genes. The phenotype of mct8

mutants was compared with mct8 morphants showing no discrepancies. The results observed in mct8 knockout model suggest that the MT3 disruption lead a loss of neural progenitor cells and consequently loss of neurons with an increased cell proliferation. The results verified in thraa knockout model suggest that the SC cytoarchitecture is altered in thraa mutants. The two knock-out models correlation shown that the dorsal cell population of the SC appear to be highly influenced by MT3 transcription action. The dorsal and medial cell populations seem not to depend directly on the action of the hormone, but mostly on the action of thraa as aporeceptor. Outstanding models of study allow us to make a parallel with the human neurodevelopment, increasing the knowledge about the physiopathology of Allen-Herdorn-Dudley syndrome and Thyroid hormone resistance syndrome.

SYMPOSIUM 10 – GUT MICROBIOTA

57ASM —093 | Effects of Ramadan fasting on gastrointestinal motility in healthy subjects

H. Abdallah[1]; S. Santoro[1,2]; M. Khalil[1]; I. Farella[1]; E. Lanza[1]; A. Di Ciaula[1]; P. Portincasa[1]
[1]University of Bari Aldo moro, Bari, Italy; [2]Eurisko Technolgy Srl, Modugno, Italy

Background: Previous studies documented several beneficial metabolic effects of intermittent fasting. Ramadan can be considered a model of intermittent fasting and consists of prolonged fasting practised by Muslims for 24–30 days. The effects of intermittent fasting on the gastrointestinal tract are not properly investigated. We, therefore, explored noninvasively the effects of Ramadan on gastrointestinal dynamics in healthy subjects.
Methods: 21 healthy subjects were enrolled (M: F 10:11, age 30.7 ± 1.6, BMI 23.3 ± 3.5). Fasting/postprandial gallbladder and gastric motility (Sonoscape E2 Exp by Fujifilm Ultrasound machine provided by Eurisko Technology srl and equiped with 1–7 mhz 3C-A convex probe) and orocecal transit time (lactulose H2-breath test, Medimar SrL, Milano, IT) were assessed simultaneously at baseline (T0) and after 30 days of Ramadan fasting (T30). Postprandial changes of gallbladder volume and antral area were recorded every 15 min for 2 h following ingestion of a liquid test meal (Nutridrink® Nutricia SrL, 200 mL, fat 13.4 g, 300 Kcal).
Results: Compared to baseline, a significant reduction at T30 occurred with fasting volume (from 22.4 ± 4.7 to 20.9 ± 4 mL, $p = 0.006$), postprandial residual volume (from 9.3 ± 2.4 to 6.8 ± 2.1 mL, $p = 0.0002$), gastric half-emptying time (from 67.1 ± 12.1 to 57.8 ± 9.4 min, $p = 0.007$), and OCTT (from 105.7 ± 16.6 to 84.8 ± 5.3 min, $p = 0.0006$). Gallbladder refilling rate increased at T30 (from 0.47 ± 0.15 to 0.67 ± 0.11 mL/min, $p < 0.0001$).
Conclusion: Intermittent fasting lasting for one month determines significant variations in gastrointestinal motility in healthy subjects. Further studies are needed to verify possible beneficial effects in clinical conditions characterized by defective gallbladder and gastrointestinal motility, paving the way to relevant primary prevention measures.

57ASM – 094 | The role of probiotics in children with Idiopathic Nephrotic Syndrome

I. Farella
Aldo Moro Università Bari, Sannicandro di Bari, Italy

Background: The role of immune dysregulation in the idiopathic nephrotic syndrome (INS) is widely recognized. However, the precise pathogenetic mechanisms are still unclear. T cells regulators seem to play a critical role, since they are decreased in children with INS. A major role is also possible for the interplay between gut microbiota, the development and maturation of the immune system, mainly through the production of bacterial metabolites. In this context, the administration of probiotics might positively affect the links between gut microbiota and renal function (the gut-kidney axis). This aspect, however, is still scarcely studied in INS.
Methods: We explored the role of microbiota in INS by a literature search (published articles between 1st January 2000 and 1st March, 2023) focused on the potential role of probiotics.
Results: A total of 15 clinical studies were found and 8 articles were selected according to the quality of results. Evidence reports an unbalanced gut microbiota in paediatric patients with INS, characterized by a reduced microbial diversity, a smaller proportion of butyric acid-producing bacteria and a reduced number of circulatory T cells. A unique randomized controlled trial showed a significant increase in butyrate-producing bacteria and T regulator counts in 20 INS children treated with 3 g of Clostridium butyricum, as compared with controls. Treated children also showed fewer NS relapses per year.
Conclusions: Despite the growing interest on the gut-kidney axis, the effective role of gut microbiota in INS is still poorly investigated. The few evidence concerning the role of probiotics in INS points out the direction for the development of new therapeutic approaches targeting intestinal microbiota.

57ASM – 111 | Diagnosing the gastric microbe Helicobacter Pylori (HP) infection by 13C-urea breath. Mass-spectrometry versus the novel ISOMED laser-based analysis

M. Khalil[1]; A. Citrino[2]; H. Shanmugam[1]; A. Di Ciaula[1]; L. Bonfrate[1]; R. De Veneto[1]; P. De Benedictis[1]; T. Kaariainen[3]; K. Nummila[3]; P. Portincasa[1]
[1]Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy; [2]Richen Force, China; [3]VVT, Finland

Background and Aim: An adequate diagnostic approach to HP infection can avoid the overuse of resources. The breath test exploits the high urease activity of HP to diagnose the infection, linked with an increase in 13C in exhaled air and, therefore, of the 13C/12C ratio. The new cooperation project between Technical Research Centre of Finland Ltd (VTT) and Richen Force holdings presented a new "ISOMED" prototype, an innovative laser-based analyser for measuring the isotope ratios 13C/12C and 18O/16O in 13C-urea HP breath test (UBT). The laser-based analysis might improve the accuracy of the delta-over-baseline (DOB) measurements, as compared with mass-spectrometer (MS) analysis, reducing the volume of samples and the measurement time. However, a validation of this technique is still unavailable. The aim of this study was to compare results from the ISOMED prototype with MS-UBT test as reference measurement.
Methods; 101 patients underwent UBT, and collected breath samples were analysed using both analytical techniques. Samples were collected prior (0-min) and 30-min after (30-min) administration of the 13C-urea (Richen Medical Sciences). At each time, two separate sample bags (for ISOMED and MS) were subsequently collected.
Results: Adequate samples were collected from 95 patients (6 bags discarded for defective storage). With a 3.5‰ DOB as a diagnostic cut-off value, 10 patients (10.5%) were HP(+)ve and 85 patients (80.5%) were HP(−)ve, with full agreement between MS and ISOMED ($R^2 = 0.995$; $p < 0.00001$). Results from ISOMED were comparable when using up to 100 mL of sample gas (average of 11 measurement cycles lasting 5 s) or only up to 10 mL of sample gas (single cycle measurement lasting 10 s).
Conclusions: The ISOMED laser-based prototype, compared with MS-UBT, yields accurate results, employs smaller breath samples and shorter analysis times. This innovative equipment is promising for UBT diagnosis of HP infection.

57ASM – 121 | Non-alcoholic fatty liver disease (NAFLD) and inadequate adherence to Mediterranean Diet predisposes to altered intestinal permeability

H. Shanmugam[1]; A. Di Ciaula[1]; M. D. Di Palo[1]; G. Garruti[2]; P. Portincasa[1]
[1]Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University o, Bari, Italy; [2]Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases, Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari Medical School, Bari, Italy

Background: Intestinal permeability (IP) plays a key role in preserving gut-metabolic functions in health. However, a comprehensive evaluation of IP as a marker of intestinal barrier integrity in dysmetabolic patients is still missing. We aimed to assess IP in the whole gastrointestinal tract according to non-alcoholic fatty liver disease (NAFLD), Body Mass Index (BMI), and dietary habits.
Materials and Methods: In 145 gender-matched subjects (age: 45.2 ± 1.1 years (mean ± SEM), BMI: 27.6 ± 0.52 kg/m2, NAFLD: 56.5%, obese:37.2%), liver steatosis was scored by ultrasonography (0–3). IP was measured by urinary excretion of ingested sugar probes absorbed at different gastrointestinal levels. Sucralose (2 g), Sucrose (40 g), Lactulose (5 g), and Mannitol (2 g) dissolved in 200 mL of water were used as markers for permeability at the level of stomach-duodenum, small intestine (LA/MA ratio) and colon. Urine sugar concentrations were measured by HPLC-MS (AB Analitica, Padua, Italy). Adherence to Mediterranean Diet (MD) was assessed by a validated questionnaire. Subjects were stratified according to the presence of NAFLD, BMI, and adherence to MD.
Results: The mean grade of liver steatosis in NAFLD subjects was 1.5 ± 0.06. Subjects with NAFLD were significantly older and heavier than subjects without NAFLD. Urinary sugar recovery revealed increased stomach (i.e., Sucrose recovery) and colon (i.e., Sucralose recovery) permeability in subjects with NAFLD, then in those without NAFLD. When subjects were classified according to BMI, increased stomach and colon permeability were detected in obese subjects, as compared with normal weight and overweight subjects. Subjects with inadequate adherence to MD had significantly increased stomach and small intestine permeability than those with adequate adherence.
Conclusion: Altered gastrointestinal permeability is linked with obesity, NAFLD, and unhealthy dietary habits. Further studies must evaluate the possibility of new prevention measures and therapeutic strategies in dysmetabolic patients.

57ASM – 148 | Mediterranean diet as modulator of gut microbiota and gut-liver axis: From food components to experimental models

M. Khalil

Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy

Background: Trillions of microorganisms residing in the human gut contribute directly and indirectly to the regulation of host health. Long-term dietary intake shapes gut microbiome and influences the metabolome composition. Mediterranean diet (MD) is recently emerging as healthy dietary habits by exerting multilevel health-promoting effects not only on gut microbiota, but also on local and distant organs such as liver, heart, and brain.

Aim and Methods: Recent advances have been made for studying the modulatory effects of diet and/or diet components from the Mediterranean area on the gut-liver axis. Besides our previous experimental results, we further reviewed the recent published articles regarding the modulatory effects of diet, diet components, and natural bioactive compounds from MD on cells, animals, and human experimental models.

Results: Plant-based MD includes mainly vegetables, legumes, fruits, olive oil and nuts and provide a high amount of fibre, polyunsaturated fatty acids, polyphenols, and vitamins with evident antioxidative, anti-inflammatory, and probiotic-like properties in humans. Cellular and animal models provided a deeper information concerning the mechanisms involved in the beneficial effects of MD, focusing on the activated pathway by commensal microbial communities and their metabolites such as short-chain fatty acids (SCFA). Different cellular and animal models have been developed to study the interaction between gut and liver, in particular, co-culture (intestinal and hepatic cells), LPS-insulted hepatic and Kupffer cells, and 3D bacterial cellular models. High-fat rich diet is manly used for the study of gut-liver alteration in animals. In these models, the effects of components from MD have been documented and the mechanism of action of some of them is clarified.

Conclusion: Despite the poor information about the action mechanism, studies on cells, animals, and humans revealed the combined role of MD in toto or single components as an effective strategy for treating disorders within the gut-liver axis.

57ASM – 153 | Uncovering a candidate gut miRNA role in mitochondrial dysfunction and inflammation: Impact on Parkinson's disease

B. Guedes[1]; S. Duarte[1,2]; S. Morais Cardoso[1,3]; A. R. Esteves[1,2]

[1]Center for Neuroscience and Cell Biology, Coimbra, Portugal; [2]IIIUC-Institute of Interdisciplinary Research, University of Coimbra, Coimbra, Portugal, Coimbra, Portugal; [3] Institute of Cellular and Molecular Biology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, Coimbra, Portugal

Introduction: Parkinson's disease (PD) is a multisystemic neurodegenerative disorder characterized by the progressive loss of midbrain dopaminergic neurons which results in the appearance of cardinal motor features. Remarkably, the onset of motor symptoms is preceded by a long prodromal phase in which early gastrointestinal pathology occurs. Several reports demonstrated an altered gut microbiome composition in PD patients. Evidence suggests that gut microbiota has an important role in the gut-brain axis and are linked with PD progress and onset. The mechanisms through which the gut microbiota regulates brain function remain poorly understood; however, microRNAs have been highlighted has important players. In fact, a recent study showed an enrichment of submucosal miRNA-486–5p in colonic biopsies from PD patients supporting this miRNA involvement in PD onset and progression.

Material and Methods: Our major goal was to investigate if this miRNA could contribute to PD pathogenesis by affecting mitochondrial function and activating inflammatory responses. To do so, in vitro effects were evaluated by transfecting Caco-2 cells and SH-SY5Y cells with miRNA-486-5p.

Results: We found that miRNA-486-5p overexpression downregulated IL-23 receptor levels leading to a decrease in IL-17 levels and an increase IL-1β levels which triggered the loss of intestinal permeability as observed by a decrease in ZO-1 and occluding levels. In SH-SY5Y cells we detected that miRNA overexpression significantly reduced cardiolipin levels which was correlated with an increase in ROS levels contributing to the activation of inflammatory responses, as observed by IL-1β, NF-κB and Caspase-1 increased levels.

Conclusions: Overall, we conclude that miRNA-486-5p contributes to gut inflammation and loss of the gut barrier integrity in Caco-2 cells whereas in SH-SY5Y cells leads to the activation of inflammatory responses due to mitochondrial dysfunction. This study paves the way to clarify the contribution of gut miRNAs in inflammation and mitochondrial dysfunction in PD development.

57ASM – 157 | Microbiota fermentation of ingested inulin as true marker of orocecal transit time, as compared with lactulose

E. Lanza; A. Di Ciaula; P. Portincasa
Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy

Background: Hydrogen breath test (H2BT) following the ingestion of the non-absorbable disaccharide lactulose is frequently used to measure orocecal transit time (OCTT), due to substrate fermentation by the proximal colon microbiota. However, 10 g lactulose itself may accelerate OCTT due to an osmotic effect in the gut lumen, becoming an unrealistic expression of the true transit speed. The present study compared OCTTs following the ingestion of lactulose or inulin, an oligosaccharide almost completely fermented by the colonic microbiota but not osmotically active, due to high degree of polymerization.

Methods: Enrolled were 10 healthy subjects (mean age 28 ± 1 yrs, body mass index 22.6 ± 0.6Kg/m2) who underwent, on two consecutive days, H2BT during 4 hrs after ingestion of lactulose or inulin in a random fashion. Differences were compared by Student's t-test for paired data.

Results: Lactulose-OCTT was about 2.5-times faster (123.5 ± 7.1 min SEM), as compared with inulin-OCTT (311.1 ± 23.5 min, $p = 0.00001$). The average time difference (delta time) between the two tests was 187.6 ± 17 min, with large inter-individual variability (range 118–265 min). Thus, a linear regression analysis was performed between the delta-time value recorded in each subject and the OCTT measured with lactulose or inulin. This correlation was stronger in the case of inulin ($R^2 = 0.975$, $p < 0.01$) than lactulose ($R^2 = 0.73$, $p > 0.01$), possibly due to a higher inter-individual fermentation variability and osmotic properties following lactulose, as compared with inulin.

Conclusions: Microbiota fermentation of inulin is a precise tool to obtain a more realistic measurement of OCTT, as compared with lactulose. An additional benefit seems a lower inter-individual fermentation variability following inulin, than lactulose. Additional studies should verify the combination of the inulin breath test with other non-invasive tools (as ultrasound) to comprehensively explore the gastrointestinal dynamics at different levels of the gastrointestinal tract. The role of gut dysbiosis/small intestinal bacterial overgrowth on indices of intestinal transit remains largely unexplored.

57ASM – 185 | Phenotyping the obesities or precision medicine in obesity

G. Fruhbeck
Department of Endocrinology & Nutrition, Clínica Universidad de Navarra; CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), ISCIII; Obesity and Adipobiology Group, Instituto de Investigación Sanitaria de Navarra (IdiSNA); Metabolic Research Laboratory, Clínica Universidad de Navarra, Pamplona, Spain

Background: Obesity is a frequent, chronic, multifactorial and relapsing disease process. Its prevalence has increased over the past decades imposing a huge burden on economies, individuals, and society at large. Thus, excess weight and its accompanying comorbidities embody one of the main health problems worldwide. Despite huge efforts and advances in scientific understanding, to achieve successful and sustained weight loss has been a continuous medical challenge. The cumbersome management of obesity is, at least in part, due to a heterogenous underlying pathophysiology being further influenced by multiple external and socioeconomic factors. In this line, the gut microbiota as well as in utero, and intergenerational effects, epigenetics, sleep curtailment, endocrine disruptors, and ambient temperatures, among others, also need to be contemplated.

Several precision medicine initiatives have been proposed to improve obesity outcomes. Whilst most of these initiatives in other medical fields are based on -omics technologies, pathophysiological and behavioural phenotypes can be also used to better classify patients according to their specific characteristics. The ability to quantify aspects related to energy balance has led to the identification of specific adipocyte, gastrointestinal and behavioural traits that differ between people living with obesity. The diverse quantitative and qualitative physiologic traits regarding energy homeostasis, appetite, satiety, thermogenesis, lipolysis, energy storage together with pathophysiological features related to cardiometabolic risk and psychological-behavioural characteristics should be explored in people living with obesity since they have the power to translate into a more immediate clinical impact.

Conclusion: Capturing the full phenotypic picture of people living with obesity would provide more clinically relevant and useful information. In this context, a phenotype-guided approach could probably attain better therapeutic outcomes.

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57ASM – 186 | Gastrointestinal effects of different foods: Combined use of functional ultrasonography and perception scores

A. Di Ciaula; J. Schmidt; E. Lanza; H. Shanmugam; I. Farella; P. Portincasa

Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo, Bari, Italy

Background: Nutraceuticals are increasingly employed as preventive and curative tools. However, standardized approaches to measure agreeability and functional gastrointestinal effects of ingested food are still lacking.

Methods: We combined validated ultrasonographic techniques (gastric and gallbladder emptying), hydrogen breath test (orocecal transit time, OCTT) and questionnaires / visual analogue scales (VAS, quantification of sensory perception) in 114 healthy subjects ingesting 6 different types meals: liquid (Nutridrink®, Nutricia SrL, 200 mL, fat 13.4 g, 300 Kcal) [NUT] or mixed (80 g white bread [WBR], 80 g pasta [PAS], 24 g of almond cultivars Cea [ACE] and Californian [ACA], 27 g of dark chocolate [CHO]). Tap water was added to each food to reach a final isovolumetric meal (215 mL). All tests were performed during 2 h.

Results: Pasta induced the slowest gastric emptying (half-emptying time 57.9 ± 4 min, vs. 33.5 ± 2.1, 47.2 ± 2.7, 33.4 ± 2.8 and 41.9 ± 1.9 following Nutridrink®, bread, almonds and chocolate ingestion, respectively; $p < 0.05$). The smallest postprandial gallbladder volume was recorded following Nutridrink® (8.5 ± 0.6 mL vs. 11.4 ± 1.5, 12.0 ± 1.1, 11.0 ± 0.8, 10.3 ± 0.6 following pasta, bread, almonds and chocolate, respectively, $p < 0.05$). OCTT values were comparable between all foods. Inter-meal variability in gallbladder and gastric motility indices were mainly secondary to the different composition (mainly calorie and fat content) and physical state (liquid vs. solid) of meals. Perception scores allowed an accurate assessment of organoleptic characteristics of bread, almonds and chocolate and quantification of individual agreeability of each food.

Conclusions: The combination of multiple techniques in a same day is a feasible tool to comprehensively assess and quantify gastrointestinal and gallbladder motility following ingestion of specific liquid or solid foods. This approach allows comparative motility studies, organoleptic assessment and quantification of individual agreeability, providing useful information about the effects of specific nutraceuticals in health and disease.

57ASM – 201 | Differences in the characteristic of gut microbiota and metabolome in adult subjects with healthy and unhealthy obesity

N. Serale[1]; F. M. Calabrese[1]; M. Vacca[1]; G. Celano[1]; V. A. Genchi[2]; S. Perrini[2]; F. Giorgino[2]; L. Gesualdo[3]; M. De Angelis[1]

[1]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy; [2]Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases – Department of Precision and Regenerative Medicine and Ionian Area University of Bari Aldo Moro, Bari, Italy; [3]Nephrology, Dialysis and Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari Aldo Moro, Bari, Italy

Introduction: Obesity, an urgent and growing global public health threat, is a significant risk for cardio-metabolic diseases. Studies suggest that metabolically healthy obese subjects are at lower risk of cardio-metabolic diseases than metabolically unhealthy obese ones. To date, the role of microbiota in the development of these two different obesity phenotypes is still unknown. The aim of this study was to characterize gut microbiota and metabolome composition and functionality in metabolically healthy and unhealthy obese adult subjects considering their clinical data and dietary conditions.

Materials and Methods: An observational study was conducted on healthy controls and metabolically healthy and unhealthy obese subjects. Blood samples and food questionnaires were collected. The gut microbiota was characterized in faecal samples through quantitative PCR. Gas chromatography coupled with mass spectrometry was performed for the analysis of untargeted metabolites and short-chain fatty acids.

Result: The amount of Clostridium coccoides was lower in metabolically unhealthy obese than in healthy controls (q-value = 0.0003). Lactobacillus genus (q-value = 0.015) and Lactiplantibacillus plantarum (q-value = 0.015) species were higher in metabolically healthy obese subjects, while Prevotella (q-value = 0.03), Desulfovibrio (q-value = 0.02), and Lactiplantibacillus plantarum (q-value = 0.04) enriched the microbiome of metabolically unhealthy obese. The level of Acetone, beta-Myrcene, Tetradecane, 1,5,9-Undecatriene-2,6,10-trimethyl, 7-Hexadecanol, Estragole, alpha-Terpineol, Undec-6-en-2-one, 5,9-Undecadien-2-one-6,10-dimethyl, 2-Piperidinone, Nonanoic acid, 2,4-Ditertbutylphenol, and 6-Pentadecen-1-ol metabolites were significantly different among the three groups. In addition, a lower presence of Butanoic acid was observed in metabolically unhealthy obese.

Conclusion: Lower proportions of Lactobacillus genus and Clostridium coccoides, higher proportions of

Prevotella, Desulfovibrio, and Lactiplantibacillus plantarum, and metabolome diversity could be indicators of metabolic unhealthy obesity. These differences in microbiota and metabolome in adults with healthy and unhealthy obesity suggest that some characteristics of gut microbiota and metabolome could be involved in the link between morbid obesity and cardio-metabolic diseases.

57ASM – 226 | The market for probiotic supplements in Italy and Europe: A continuous rise documenting a consolidated therapeutical efficacy

C. Greco[1,2]; A. Di Ciaula[2,3]; M. Khalil[3]; L. Bonfrate[3]; N. Messina[1]; P. Portincasa[3]
[1]Omega Pharma srl, Cantù, Italy; [2]PhD Program Health Sciences, Clinical Medicine and Oncology, University of Bari, Italy; [3]Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari Aldo Moro, Bari, Italy

Background: Probiotics are frequently used to induce gastrointestinal and systemic beneficial effects counteracting gut dysbiosis. The commercial burden of this therapeutic tool, however, is still partly unknown.
Methods: we used data from Ipa Europe (International probiotics association) and IQVIA (market research information) to explore the European market for probiotics in the years 2021–2022 and estimates up to 2026.
Results: The European market for probiotics reached 1.464 billion euros in 2021, close to 25% of the world value, with an expected growth rate of 3% in the period 2021–2026. Italy is the third largest market in the world (560.5 million euros in probiotic sales in 2021, expected growth of 5% in the period 2021–2026). Italy, Germany and France together represent the 64% of EU sales, with a market of 775.6 million euros. An IQVIA survey on probiotics sales in Italian pharmacies showed a growth even in the last fraction of 2022. The final balance for the last year shows an increase of 13.3% in value and 11.7% in volume compared to 2021. The Top Performers sold 8.9 million pieces in 2022 (28.1% of the total market), as compared to 7.3 million pieces in 2021 (the 25.7% of the total market), with a growth of 22.5% per unit and 24.6% in value (2022: 143.8 million € vs. 2021: 115.5 million €). Among the best-known brands, emerge Enterolactis, Lactoflorene, Vsl3, Yovis, Enterelle, Reuflor and Dicoflor. The Prolactis lines emerge for the innovative and patented pharmaceutical technologies (microencapsulated probiotics based on Bifidobacterium Breve, Lactobacillus Rhamnosus, group B vitamins and FOS).
Conclusions: Despite the economic crisis, the last year revealed a significant rise in the probiotic market, possibly due to the therapeutical efficacy of this approach in the management of a number of high-prevalence diseases.

57ASM – 227 | Pasta enriched whit de-oiled wheat "germ" as an innovative functional food

M. Vacca[1]; A. Annunziato[1]; M. Calasso[1]; E. Lanza[2]; I. Farella[2]; L. Bonfrate[2]; A. Di Ciaula[2]; M. Greco-Miani[3]; P. Portincasa[2]; M. De Angelis[1]
[1] Department of Soil, Plant and Food Sciences (DiSSPA) and [2]Clinica Medica "A. Murri", Department of Preventive and Regenerative Medicine and Ionian Area (DiMePrev-J), University of Bari "Aldo Moro", Bari, Italy; [3]Casillo Next Gen Food SrL, Corato, IT

Background: Pasta is a key component of Mediterranean diet, is a cereal-based food ideal for affordability, palatability, and nutritional quality. Also, wheat germ is one of the most important by-products of the milling industry. We compared several properties of 5 types of pasta looking for an ideal functional food.
Methods: Batches of pasta consisted of (1) control (water and semolina dough with the addition of integral-like colouring agent) (CP) and dried pastas with addition of (2) 30% de-oiled durum wheat "germ" (EP1); (3) 30% "bran" de-oiled wheat (EP2); (4) 27% de-oiled wheat "germ" plus 6% microencapsulated durum wheat "oil" (EP3); and (5) 27% de-oiled wheat "bran" plus 6% microencapsulated durum wheat "oil" (EP4).
We performed chemical characterization, assay of total phenols and antioxidant activity, simulated digestion prior to faecal microbiota fermentation, and in vivo palatability cooking test.
Results: Carbohydrates and protein content was similar, while fibre content was higher across all EPs, compared to CP. EPs had significantly higher concentration of total phenols and an improved scavenging activity than CPs. EP1 and EP3 had the highest ($p < 0.05$) values of both phenols and scavenging activity with a trend for EP3 for highest values. In vitro, EP1 and EP3 showed an increased viability and density of various gut microbial patterns, in particular lactic acid bacteria and bifidobacteria. The palatability study showed that 9 min cooking time was ideal to disclose the highest score in EP1 and EP3.
Conclusions: We explored the health promoting properties featuring the innovative pasta containing de-oiled wheat "germ". Starting from the promising outcomes from in vitro assays, especially EP1 and EP3 deserve further studies in vivo as novel functional foods supporting healthy properties.

57ASM – 237 | Focus on: The link between infections and sarcopenia in internal medicine patients

G. Imbimbo
Sapienza University of Rome, Italy

Sarcopenia is a condition characterized by the loss of muscle mass and its function. The prevalence of sarcopenia increases with ageing and with the number of comorbidities. The presence of sarcopenia in patients hospitalized in Internal Medicine wards is very common and has a negative impact on clinical outcomes, determining mainly reduced survival and higher incidence of complications, including infections. Patients hospitalized in internal medicine setting may be affected by sarcopenia before, during or after their admission in the hospital. In particular, sepsis and chronic infections are common causes of sarcopenia due to the underlying inflammatory stimuli that may lead to muscle derangements. On the other side, sarcopenia itself represents a risk factor for the occurrence of infections, including pneumonia. For this reason, maintaining and/or improving muscle mass and nutritional status through multidisciplinary and personalized interventions represent a promising approach to reduce several complications, including systemic infections and improve the outcomes of patients hospitalized in internal medicine wards.
Recent and robust clinical data on this topic will be analyzed and discussed.

57ASM – 238 | Focus on: Impact of plant-based protein sources on gut microbiota

M. Andreini
Sapienza University of Rome, Italy

Among the several endogenous and exogenous factors that have been implicated in modulating gut microbiota, the diet has emerged as pivotal in driving modification of its composition and function. Importantly, plant-based diets, characterized by low frequency of animal food consumption, have been increasingly recommended for supposed health benefits and for ethical aspects. However, the impact of plant-based diets and in particular protein sources on gut microbiota are matter of investigation. It has been reported that well-planned vegan diets and their associated components (mainly fibers and polyphenols) affect both the bacterial composition and metabolic pathways of gut microbiota. Also, demanding for plant-based meat alternatives in order to replace animal meat is increasing and whether this type of alternative protein sources may impact on gut microbiota composition and function - due to its potential "ultra-processed" nature - is matter of discussion.
How plant-based protein sources promote either eubiotic or dysbiotic changes in gut microbiota should be better clarified in clinical studies and the most updated data available in the literature on this topic will be discussed.

SYMPOSIUM 11 – BIOINFORMATICS

57ASM – 019 | Unveiling the role of moderate exercise training in immune system and prostate signalome: data from a rat model

E. Nascimento-Gonçalves[1,2]; F. Seixas[3]; C. Palmeira[4]; G. Martins[4]; C. Fonseca[1]; J. A. Duarte[5]; **A. Faustino**[1]; B. Colaço[3]; M. J. Pires[1]; M. J. Neuparth[5]; D. Moreira-Gonçlaves[5]; M. Fardilha[6]; M. C. Henriques[6]; D. Patrício[6]; S. Pelech[7]; R. Ferreira[2]; P. A. Oliveira[1]
[1]CITAB, Inov4Agro, UTAD, Vila Real, Portugal; [2]LAQV-REQUIMTE, UA, Aveiro, Portugal; [3]CECAV, AL4AnimalS, UTAD, Vila Real, Portugal; [4]Clinical Pathology Department, IPO, Porto, Portugal; Experimental Pathology and Therapeutics Group, Portuguese Institute of Oncology, Porto, Portugal; [5]CIAFEL, FADEUP, Porto, Portugal; [6]Department of Medical Sciences, iBiMED - Institute of Biomedicine, UA, Aveiro, Portugal; [7]Department of Medicine, University of British Columbia, Vancouver, B.C., Canada; Kinexus Bioinformatics Corporation, Suite, Suite, Canada

Background: Prostate cancer (PCa) is among the most prevalent cancers worldwide. This work aimed to understand the effects of exercise training in a rat model of PCa chemically and hormonally-induced.
Material and Methods: Fifty-five male Wistar rats were divided into: control sedentary (SED+CONT; $n=10$), control exercised (EX+CONT; $n=10$), induced sedentary (SED+PCa; $n=15$) and induced exercised (EX+PCa; $n=20$). Exercised animals were trained in a treadmill for 53 weeks. PCa induction consisted in a multistep protocol including flutaminde and N-methyl-N-nitrosourea administration, followed by testosterone propionate implants. At sacrifice, dorsolateral prostate lobe and peripheral blood was collected. Data were analysed using SPSS 25 and values were statistically significant at $p<0.05$.
Results: CD4+/CD8+ ratio in dorsolateral prostate tissue was decreased in EX+PCa group when compared to SED+PCa group. Peripheral levels of γδ T cells were higher in exercised groups ($p<0.05$). The most prominent changes in prostate proteome induce by exercise were Estrogen Receptor-alpha (ERalfa; ESR1) upregulation of

stimulatory Ser-104 phosphorylation (+ 1976% change from SED+PCa rats) and Mitogen-activated Protein Kinase 13 (MAPK13; p38δ MAPK) downregulation of stimulatory Thr-180 and Tyr-182 phosphorylation (-80% change from SED+PCa rats).

Conclusions: Our results reinforce the beneficial role of exercise in anti-tumour immune response, with modulation of ER-alpha and MAPK pathways and remodelling of peripheral lymphocyte subpopulations, and lymphocyte infiltration in prostate tissue during carcinogenesis.

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57ASM – 091 | The future of diabetes research: Advancements in risk factors and characteristics

R. Silva[1]; A. P. Januário[1]; M. J. Meneses[1,2]; J. F. Raposo[2,3]; M. P. Macedo[2,3]; A. Romão[1]

[1]DECSIS II Iberia, Évora, Portugal; [2]iNOVA4Health, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisbon, Portugal; [3]APDP – Diabetes Portugal – Education and Research Center, Lisbon, Portugal

Introduction: The estimated prevalence of diabetes in the European population (20–79 years old) was ~10% in 2021. As the age structure of the European population ages, these prevalence's may evolve. The aim of this study was to identify the profile, main related risk factors of a person with prediabetes or type 2 diabetes and verify the association of clinical and sociodemographic factors, seeking to discuss treatment and support guidelines.

Material and Methods: PREVADIAB2 cohort was used to assess which clinical factors (glucose, insulin, free fatty acids and C-peptide, at fasting and 30 and 120 min of the OGTT) and sociodemographic and anthropometric factors are associated with a person with diabetes and prediabetes. Two multivariate logistic models were developed, whose response variable assumes a value of 1. The diabetes classification was based on IDF/WHO criteria and through a decision tree underlying the European standard to assess a structure that is even more sensitive to the characteristics of the studied Portuguese population. Multivariate cluster analysis techniques were used to analyse clinical and sociodemographic characteristics broken down by gender.

Results: Of all the variables presented, only the c-peptide (0 and 30 min), free fatty acids (30 min), insulin (120 min) and glucose (30 min) are directly associated with the patients' classification of diabetes and prediabetes (ROC: 0.897; sensitivity: 77.25%; specificity: 87.22%; accuracy: 84%) adjusted by Hosmer–Lemeshow (p-value = 0.312). For gender, three distinct groups were formed using only c-peptide, free fatty acids and insulin. Considering the sociodemographic information, the groups did not present significant differences.

Conclusion: Our data shows that a comprehensive evaluation of patients with diabetes must go beyond glucose and include C-peptide, free fatty acids, and insulin levels. These factors play a fundamental role in understanding the patient's profile and providing the most appropriate care, including early diagnosis and management.

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57ASM – 097 | Long-term spaceflights alter the biological clock in mammals and result in circadian alterations of skeletal muscle genes resembling aging-related phenotypes

D. Malhan[1,2,3]; M. Yalçin[1,2,3]; B. Schoenrock[4]; D. Blottner[4,5]; A. Relógio[1,2,3]

[1]Institute for Systems Medicine and Faculty of Human Medicine, MSH Medical School Hamburg, Hamburg, Germany; [2]Institute for Theoretical Biology (ITB), Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; [3]Molecular Cancer Research Center (MKFZ), Medical Department of Haematology, Oncology, and Tumour Immunology, Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; [4]Institute of Integrative Neuroanatomy, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; [5]Neuromuscular System and Neuromuscular Signalling, Berlin Center of Space Medicine and Extreme Environments, Berlin, Germany

Introduction: In mammals, the circadian clock plays a central role in the regulation of biological processes such as metabolism. Skeletal musculature is the largest and one

of the most dynamic and plastic organs of the human body that also exhibits circadian rhyth Alterations in circadian rhythms are among the major characteristics of aging on Earth and in manned spaceflight, leading to muscle atrophy. Molecular insights into spaceflight-related alterations of circadian regulation in skeletal muscle are missing. Whether alterations in clock genes due to spaceflight resemble aging on Earth remains unknown.

Methods: We gathered 28 published omics datasets obtained from skeletal muscle tissue or cell samples. Rhythmicity analysis, differential rhythmicity, and differential expression analysis were carried out.

Results: Core clock genes like RAR-related orphan receptor c (Rorc) and muscle atrophy-associated genes like Activating transcription factor 4 (Atf4) showed downregulation due to long-term spaceflight and aging on Earth in mammalian skeletal muscle. Finally, we will discuss common molecular alterations underlying spaceflight and aging on Earth, and how external factors such as physical exercise or 24-h fasting may compensate for the circadian disruption observed during spaceflights.

Conclusion: Our study supports the hypothesis of microgravity-triggered circadian clock changes in muscle-related pathways. Future experiments will be needed to further validate our findings and explore more insight into the circadian regulation of gene expression resulting from spaceflight using time-course experiments.

ASM – 171 | Plasma activated medium in bladder cancer treatment: Can we make it easier?

E. Tavares Da Silva[1,2,3,4]; J. Craveiro[2,4]; E. Pereira[2,3,4]; A. Salomé Pires[2,3,4]; A. M. Abrantes[2,3,4]; A. Figueiredo[1,2,3,4]; M. F. Botelho[2,3,4]

[1]Centro Hospitalar E Universitário De Coimbra, Coimbra, Portugal; [2]Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [3]Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; [4]Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal

Bladder cancer is a significant global health concern, ranking 10th in terms of incidence and 13th in terms of mortality. Plasma, a partially ionized gas consisting of ions, electrons and neutral particles, has shown promise in medical applications. Cold atmospheric plasma, which is produced at temperatures below 40°C, has been demonstrated to have an antitumor effect against several types of cancers when applied directly or indirectly to cells and tissues through a water-based liquid known as plasma activated medium. The primary mechanism underlying this effect is the production of reactive oxygen and nitrogen species, which cause an imbalance in oxidative stress and selectively treat neoplas thermal nonequilibrium plasma (produced at temperatures above 40°C) is currently used in transurethral resection of bladder cancer, an electrosurgical procedure based on thermal ablation, and emerges as a great option to produce plasma activated medium. We aim to determine the level of reactive oxygen and nitrogen species activity in 0.9% NaCl saline solution activated with thermal nonequilibrium plasma.

We exposed 0.9% NaCl to thermal nonequilibrium plasma produced by a resectoscope during short periods of time (30, 60, 120, 180 seconds), creating plasma activated medium. The reactive oxygen and nitrogen species activity was evaluated using the OxiSelect™ RONS Assay Kit.

Results showed a highly significant increase in reactive oxygen and nitrogen species activity in all exposure times when compared to the control.

These results pave the way for further studies, such as evaluating the biological effect of thermal nonequilibrium plasma-activated medium on bladder cancer cell lines. Considering its local production capacity, this solution could serve as an alternative or adjuvant in the treatment of bladder cancer as a new intravesical therapeutic tool.

57ASM – 191 | Evaluation of extracellular vesicle proteome for the diagnosis and prognosis of diffuse large B-cell lymphoma

R. Matthiesen; P. Gameiro; A. Henriques; C. Bodo; M. C. Strano Moraes; B. Costa-Silva; J. Cabeçadas; M. Gomes da Silva; H. Ch. Beck; A. S. Carvalho

Nova Medical School, Lisbon, Portugal

Introduction: The aggressive B cell lymphoma diffuse large B cell lymphoma (DLBCL) exhibits heterogeneous behaviour and requires the development of more precise biological characterization, monitoring, and prognostic tools. Extracellular vesicles (EVs) are secreted by all cell types and are currently established to some extent as representatives of the cell of origin. The present study characterized and evaluated the diagnostic and prognostic potential of plasma EV proteome in DLBCL by using state-of-the-art mass spectrometry.

Materials and Methods: In this study, we characterized the EV plasma proteome and morphology from 32 patients diagnosed with DLBCL and fifteen age-matched healthy donors.

Results: The EV proteome is strongly affected by DLBCL status, with multiple proteins uniquely identified in the

plasma of DLBCL. A proof-of-concept classifier resulted in highly accurate classification with sensitivity and specificity of 1 when tested on the holdout test data set. On the other hand, no proteins were identified to correlate with non-germinal center B-cell like (non-GCB) or GCB subtypes to a significant degree after correction for multiple testing. However, functional analysis suggested that antigen binding is regulated when comparing non-GCB and GCB. Survival analysis based on protein quantitative values and clinical parameters identified multiple EV proteins as significantly correlated to survival.

Conclusion: Plasma extracellular vesicle proteome distinguishes healthy donors from DLBCL cancer patients and holds potential EV protein markers for survival prediction. However, larger follow-up studies are required.

57ASM – 206 | Studying the mechanisms of human disease through large scale data analysis

P. Beltrao
ETH, Zurich, Switzerland

The number of genetic studies of human traits and diseases has grown over the past years with many thousands of traits analysed by genome-wide association studies or clinical studies. However, connecting trait associated genetic variation to mechanisms through individual proteins and cellular mechanisms remains a challenge. Our group is interested in building computational pipelines that aim to address this challenge. As an example, we have been developing resources that use cutting-edge AI methods to facilitate the interpretation of protein missense variants. To illustrate this, I will present work on the application of AlphaFold2 in predicting structures of human protein complexes in order to study interface disease related mutations. In parallel, we have been using interaction network-based methods to expand and prioritize trait associated genes for over 2000 traits influenced by common and rare mutations, showing how this recovers known disease genes and drug targets. The similarity of network expansion scores identifies groups of traits likely to share a common genetic basis as well as the biological processes underlying this. Through this work we can identify protein complexes and pathways linked to specific diseases or those that influence a large number of human traits. We aim to develop easy to use computational resources that can improve the workflow of clinicians and welcome suggestions on what this community need. We are collaborating with clinicians on specific applications, with a particular focus on rare disorders to facilitate the classification of variants of unknown significance and prioritization of novel disease genes.

57ASM – 221 | TimeTeller: Profiling circadian rhythms to improve performance, prevent disease and optimize treatment

A. Relógio
Institute for Systems Medicine, MSH Medical School Hamburg and Charité Medical University of Berlin, Hamburg, Germany

Background: Our endogenous time generating system -the circadian clock- regulates behavioural, physiological and cellular processes. Disruptions of the clock, via for e.g., shift work, jet lag, or genetic alterations, are linked to an increased risk of various diseases like obesity, diabetes, cardiovascular diseases and cancer. Chronomedicine, the study of the effects of time on health and disease has seen an uprising as a means to enhance health and performance, and optimize treatment timing. Despite the benefits of chronomedicine, the lack of non-invasive tools for characterizing the clock limits the potential of this field.

Material and Methods: We developed and used TimeTeller, which is a non-invasive molecular/computational tool for the characterization of circadian rhythms and prediction of daily routines, including treatment timing, which allows for the implementation of chronomedicine in its various settings.

Results: Our data shows that TimeTeller can be used to profile the circadian rhythms of different subjects and based on gene expression measurements and computational predictions derived using mathematical models and bioinformatics analysis, optimal time windows for physical performance or treatment administration can be determined.

Conclusions: By aligning an individual's circadian clock with optimal times for performing daily routines, and using personal health information across lifestyle, care, and research settings, physical and mental performance, and also the effectiveness of certain therapies can be improved.

57ASM – 229 | Bioinformatics finding novel biomarkers and mechanisms of ALS using multi-omics data integration

Stefan Bonn
Institute of Medical Systems Biology, bAIome - Center for Biomedical AI, Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Hamburg-Eppendorf, Hamburg, Germany

The advent and rapid evolution of omics technologies have promised mechanistic details of development and

disease at unprecedented depth. Here we show how we integrated omics data for murine and human data to find early signatures of ALS disease, leading to the discovery of promising novel treatment avenues.

57ASM – 234 | Big data analysis for medical prognosis of type-2 diabetes patients by artificial intelligence (machine learning)

H. K. Maerz; R. U. Pliquett
Gemeinschaftslabor Cottbus, 03050 Cottbus, Germany

Medical statistics is a well known means for identifying disease-related connections, i.e. associations or causative risk factors of diseases such as type-2 diabetes. For the latter, patients collect a wealth of data using different electronic devices and sensors. The analysis of patient-related data during a long-term treatment can yield important real world evidence and affect both therapy decisions and prognosis. However, the process of gathering patient-related data is time consuming and subject to local regulations. The use of contemporary software solutions may ease the effort, enhance quality and decrease the analysis time. Securely accessible via the internet, the available data and yielded analysis results are accessed by a common browser. Over time, the data of individual patients will grow and thus increase data quality. If the amount of data is big enough for statistics, we can employ machine learning on data of interest to further improve the quality of data analysis and, ultimately, diagnosis derived from these data.

For this aim, we currently build a flexible software concept that is modular, configurable and scalable with regard to the data growth for the use of data analysis derived from all aspects of diabetes mellitus affecting a growing proportion of humans worldwide. Specifically. glucose monitoring data, food-related data, biometric information, activity logs, available laboratory results will be stored in a secure data repository offline on patient-owned data-storage device, and if data-security standards are high enough, in an internet-accessible data repository. As for concepts and technologies employed in other areas and industries, we will perform data analysis on a patient-individual level to yield individual diagnosis-relevant conclusions, i.e. risk of hyperglycemia during the day based on glucose monitoring, food and activity data. In addition, for the internet-accessible data repository, artificial-intelligence-based approaches may be relevant for public-health issues on a cohort level.

SYMPOSIUM 12 – OTHER TOPIC BASIC RESEARCH

57ASM – 004 | Antiplatelet activity of nitrated fatty acids from tomato pomace

L. Rodriguez[1]; E. Fuentes[1]; A. Trostchansky[2]; I. Palomo[1]
[1] Thrombosis Research Center, Medical Technology School, Department of Clinical Biochemistry and Immunohaematology, Faculty of Health Sciences, Universidad de Talca, Talca 3,480,094, Chile, Talca, Chile; [2] Departamento de Bioquímica and Centro de Investigaciones Biomédicas (CEINBIO), Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Introduction: In the pathophysiological process associated with cardiovascular diseases (CVD), platelet activation with the key participation of mitochondria favours the development of CVD. The finding of bioactive compounds with biological activity that help improve the nutritional status of the population constitutes a powerful tool for the prevention of CVD. Previous studies by our group have observed that tomato pomace has a potent antiplatelet activity. This activity by tomato pomace could be attributed to its high content of fatty acids (> 30%). The fatty acids present in tomato pomace can nitrate during digestion, these are the product of the reaction of nitrogen dioxide with unsaturated fatty acids. So it is important to better understand the mechanisms of the formation of nitrated fatty acids from tomato pomace and its antiplatelet action.

Material and Methods: In this study we evaluated the formation of nitrated fatty acids present in tomato pomace, as well as its antiplatelet potential to reduce cardiovascular events. Turbidimetry and flow cytometry techniques allowed us to evaluate the inhibition of platelet activation and aggregation in washed platelets when they were stimulated by thrombin receptor peptide (TRAP-6). Additionally by HPLC/MS/MS we were able to identify the nitrated fatty acids responsible for the antiplatelet potential.

Results: Extracts of nitrated tomase showed concentration-dependent antiplatelet potential when platelets were stimulated with TRAP-6. This activity was related to the presence of nitrated linoleic acid, which inhibited p-selectin expression and GPIIb/IIIa activation by flow cytometry.

Conclusion: The knowledge about the antiplatelet activity of nitrated fatty acids from tomato pomace will further develop new and more effective agents. Our proposal could be particularly relevant in the context of medicinal

chemistry, in addition to giving added value to this currently underused natural product.

57ASM – 030 | Effects of an aqueous extract of Santolina chamaecyparissus L. in mammary carcinogenesis: Data from a rat model

T. Azevedo[1]; J. Silva[1]; A. Valada[1]; L. Anjos[1]; T. Moura[1]; R. Ferreira[1]; M. Santos[1]; M J. Pires[1,2]; M. J. Neuparth[3,4,5]; A. Faustino[1,6,7]; F. Peixoto[8]; A. C. Silvestre-Ferreira[9]; F. Seixas[2,9]; T. Finimundy[10,11]; L. Barros[10,11]; M. Matos[1]; P. A. Oliveira[1,2,7]

[1]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Associate Laboratory Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production (Inov4Agro), University of Trás-Os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2]Department of Veterinary Sciences, UTAD, Vila Real, Portugal; [3]Research Center in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sports, University of Porto (FADEUP), Porto, Portugal; [4]Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal; [5]Toxicology Research Unit (TOXRUN), University Institute of Health Sciences, CESPU, Porto, Portugal; [6]CHRC, Department of Zootechnics, University of Évora, Évora, Portugal; [7]Academic Medical Centre of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [8]Chemistry Center – Vila Real (CQ-VR), Biological and Environment Department, Vila Real, Portugal; [9]Animal and Veterinary Research Centre (CECAV), Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), Vila Real, Portugal; [10]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; [11]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal

Background: Mammary cancer is one of the most frequent cancers among women worldwide. Santolina chamaecyparissus L. (SC) is a plant with multiple health benefits, including anticancer properties. This research aimed to assess the effects of SC in a rat model of mammary carcinogenesis chemically-induced.

Material & Methods: Twenty-eight female Wistar rats were divided into four groups ($n=7$): control (CTRL), supplemented (SC), N-methyl-N-nitrosourea (MNU) (MNU), and MNU + SC. At seven weeks of age, animals from groups MNU received an intraperitoneal injection of MNU (50 mg/kg). SC extract was added to drinking water (120 μg/mL) for 20 weeks. Body mass, food and water intake, humane endpoints, and mammary chains were evaluated on a weekly basis. At necropsy, tissues samples were collected for histological analysis. Furthermore, blood samples were collected to determine blood count and for serum biochemistry. Kidney and liver samples were used for oxidative stress analysis, and tumour samples were collected for gene expression studies. Data were analysed using SPSS 25 and values were statistically significant at $p<0.05$.

Results: Considering humane endpoints, two animals from the MNU group were sacrificed before the end of the experiment. SC supplementation increased the latency period and decreased mammary tumours' volume and weight. The extract had positive effects on haematological parameters, including mean platelet volume ($p=0.014$), platelet distribution width ($p<0.001$), and neutrophil-lymphocyte ratio ($p=0.026$). Significant differences were not observed in serum biochemistry (except for creatinine kinase) or in oxidative stress markers. Mammary tumours from MNU + SC group exhibited reduced expression of vascular endothelial growth factor (VEGF) ($p=0.0158$), proliferating cell nuclear antigen (PCNA) and oestrogen receptor (ER)-α ($p>0.05$).

Conclusions: Our results suggest that the SC extract may constitute a potential nonpharmacological therapeutic or adjuvant approach for mammary cancer.

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57ASM – 042 | Dual-targeting radioconjugates carrying TPP and a PSMA-binding moiety: Biological studies in prostate cancer models

M. Sobral; J. Santos; C. Fernandes; P. Raposinho; F. Mendes; A. M. Urbano; S. Mota; P. Oliveira; A. Paulo

University of Coimbra, Coimbra, Portugal

The development of new theranostic radiopharmaceuticals that target the prostate-specific membrane antigen (PSMA), which is commonly overexpressed in prostate cancer (PCa), is an area of active research. One successful approach involved the complexation of the beta minus emitter 177Lu with PSMA-617, a macrocyclic chelator containing a PSMA-binding moiety. The resulting radioconjugate (177Lu-PSMA-617) has been recently approved by the U.S. Food and Drug Administration (FDA) as a radiopharmaceutical for treating metastatic castration-resistant prostate cancer (mCRPC). However, beta minus emitters have limitations, including nephrotoxicity and resistance to beta radiation. Auger electron (AE) emitters,

such 111In, have been suggested as an alternative to overcome these limitations.

In order to increase selectivity and efficacy, while minimizing adverse health effects, we have designed dually-targeted 111In radioconjugates specifically directed to the mitochondria of PCa cells. Our strategy involved the incorporation in the PSMA-617 structure of a moiety (the triphenyl-phosphonium (TPP) group) that specifically targets this organelle, which is highly sensitive to ionizing radiation.

All new compounds were fully characterized by high-performance liquid chromatography (HPLC) and electrospray ionization-mass spectrometry (ESI-MS). Biological evaluations were carried out in the LNCaP, PC3 PIP and PC3 Flu cell lines and included the assessment of stability, cellular uptake and PSMA-inhibitory activity. Differences between groups were analysed using paired t-test and one-way ANOVA.

The 111In complexes were stable in physiologic conditions, demonstrated high uptake in PSMA-positive cells (PC3 PIP) and low internalization in PSMA-negative cells (PC3 Flu). The inhibitory activity of the "cold" compounds was confirmed using PSMA extracted from LNCaP cells.

Our preliminary results showed that the novel dually-targeted 111In-complexes exhibit properties that make them good candidates for Auger therapy of PCa.

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57ASM – 077 | Infrared thermometry for evaluation of brown adipose tissue activity

A. Vavrova; K. Bardová; P. Zouhar; M. Rossmeisl; J. Kopecký
Institute of Physiology, Czech Academy of Sciences, Prague 4, Czech Republic

Detection of heat production in individual organs or tissues is a notoriously difficult problem. A non-invasive tool for this measurement in laboratory animals is needed. UCP1 WT and KO male mice pre-acclimated at 30°C or 22°C were on the back smeared with vaseline to expose the skin and injected either with saline or norepinephrine. Infrared images were taken before and every 10 min after the application up till 60 min. Temperature of upper back region in the area of BAT and tail were evaluated. Next, B6 female mice were implanted by mini-mitters into abdominal cavity for core-body temperature detection. After 4 weeks of acclimatization at 22°C, saline, norepinephrine, isoproterenol and CL316.243 were subcutaneously applied and the core body temperature and skin temperature in BAT region using IR camera were followed up till 60 min. After the 2-week period of 8°C-acclimatization, the test was repeated. Both experiments were performed at 28°C. UCP1 WT mice exposed to room temperature exhibited increase in temperature in BAT region as compared to saline controls and UCP1 KO mice. Mice pre-acclimated to 8°C showed higher response in the change in both the core-body and upper-back temperature in BAT region. Isoproterenol and norepinephrine caused a transient increase first of the BAT temperature, which was followed by the increase in core-body temperature, while CL316.243 caused a lower, but long-lasting response in both temperatures.

Our results document that by using the IR camera we can detect BAT as a source of heat in UCP1 WT, but not UCP1 KO mice after the norepinephrine stimulation. The increase in BAT temperature precedes increase in core body temperature, using different adrenergic stimulators, consistent with the role of BAT as a source of heat. The use of IR camera enables us for a non-invasive detection of heat production.

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57ASM – 083 | Postnatal overweight-induced offspring neurodevelopment and behaviour alterations associated with synaptic imbalance, according to sex-specific vulnerabilities

A. Amaro[1,2,3]; M. Sá-Rocha[1,2,3]; D. Sousa[1,2,3]; M. F. Júnior[4]; L. Saavedra[5]; R. Mello-Gomes[4]; F. I. Baptista[1,2,3]; P. Matafome[1,2,3,6]
[1]Institute of Physiology and Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Portugal, Coimbra, Portugal; [2]Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Portugal, Coimbra, Portugal; [3]Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal, Coimbra, Portugal; [4]Department of Physiological Sciences, Institute of Biological Sciences, University Federal of Goiás, Goiânia, Brazil, Goiânia, Brazil; [5]Laboratory of Secretion Cell Biology, Department of Biotechnology, Genetics and Cell Biology, State University of Maringa, Brazil, Maringa, Brazil; [6]Polytechnic University of Coimbra, Coimbra Health School (ESTeSC), Coimbra, Portugal

Introduction: The first periods of life are crucial to modulate offspring neurodevelopment and behaviour. Disturbances during the postnatal period can have long-lasting effects and lead to emotional and cognitive impairments in later life. However, it is not clear the underlying

mechanistic pathways involved in these alterations. Our aim was to evaluate the effect of postnatal hyperphagia on neurodevelopment and behaviour, and the potential underpinning mechanisms involved in these alterations.

Materials and Methods: To study the postnatal hyperphagia effect in both male and female offspring, small litter procedure was performed (reduction to 3 pups at postnatal day (PND3)). During the first postnatal days (PND5 to PND17), neurodevelopment tests were conducted, and at PND44 the Elevated Plus Maze test was performed to evaluate anxious-like behaviour. During the experiment, body weight was weekly monitorized. On the day of the sacrifice – PND45 the hippocampus and prefrontal cortex (PFC) were collected for molecular analysis.

Results: Only male offspring presented an increased body weight gain. Only females presented a delay in auditory acquirement, eye-opening day and impairment in vestibular system development. Regarding locomotion ability, male offspring presented a delay, while female offspring did not show alterations. Both offspring had a worse performance in Wire Suspension Test. During adolescence, both offspring remained less time exploring open arms, denoting anxious-like behaviour. In male hippocampus and PFC, GABAA receptor presented higher levels, without alteration on PSD95 and (vesicular glutamate transporter 1) vGLUT1 levels. Similar alterations were observed in female PFC, whereas, in the hippocampus, lower (vesicular GABAA transporter) vGAT and GABAA receptor levels were detected, without alterations at vGLUT1 and PSD95 levels.

Conclusion: Postnatal overweight modulates offspring neurodevelopment, being female offspring more susceptible to neurodevelopment delay. In adolescence, offspring presents an anxious-like behaviour, associated with synaptic imbalance, according to brain region-specificities.

57ASM – 085 | Molecular characterisation of cutaneous melanoma

I. Nunes[1,2,3]; C. Costa[4]; C. Martin[1,2,5,6]; M. I. Andrade[7]; A. João[8]; J. M. Lopes[1,2,4,9]; C. Barata[10]; P. Soares[1,2,9]; H. Pópulo[1,2,9]

[1]Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal; [2]Instituto de Investigaçao e Inovaçao em Saúde (i3S), Universidade do Porto (UP), Porto, Portugal; [3]Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Instituto Politécnico de Lisboa (IPL), Lisboa, Portugal; [4]Department of Dermatology, Hospital de Sao Joao, Porto, Portugal; [5]Instituto de Ciencias Biomédicas Abel Salazar (ICBAS), Universidade do Porto (UP), Porto, Portugal; [6]Department of Dermatology, Hospital Distrital de Santarém, Santarém, Portugal; [7]Department of Pathology, Hospital Distrital de Santarém, Santarém, Portugal; [8]Department of Dermatology and Venereology, Hospital de Santo António dos Capuchos, Centro Hospitalar Universitário de Lisboa Central, Lisboa, Portugal; [9]Faculdadede Medicina da Universidade do Porto (FMUP), Porto, Portugal; [10]Instituto de Sistemas e Robótica, Instituto Superior Técnico (IST), Lisboa, Portugal

Introduction: Cutaneous melanoma (CM) is the least frequent but deadliest kind of skin cancer, highlighting the aggressiveness of these tumours. As a result of prolonged exposure to UV radiation, CM harbours one of the highest mutation rates among all malignancies. The three most common mutations occur in BRAF and NRAS genes, both important for the mitogen-activated protein kinase pathway upregulation, and in the telomerase reverse transcriptase promoter, which is linked to a poor prognosis. Although current therapies have improved patients' overall survival, low response rates and treatment resistance remain as major challenges for melanoma treatment. Therefore, melanoma investigations have shifted their interest to microRNAs (miRNAs), like miR-125a, miR- 155 and miR- 579, noncoding RNAs that affect the hallmarks of cancer and are potential melanoma biomarkers.

Project Aim: To study the molecular alterations of melanomas, to correlate them with the patients' clinicopathological data and follow- up and to identify possible prognostic and/or therapeutic biomarkers.

Materials and Methods: The mutational status analysis was performed by the Sanger method in primary tumours, metastatic lymph nodes and metastases collected at Hospital de Santarém and Hospital dos Capuchos. Whereas miRNA expression, from primary tumours collected at Hospital de Santarém, was analysed by RT-PCR.

Results: The median age of the studied series was 65 years, where most.

57ASM – 101 | Screening of neuroprotectors in the endothelin-1 focal cerebral ischemia model

R. Khazipov[1]; J. Levedeva[2]; G. Burkhanova-Zakirova[2]; K. Chernova[2]; A. Zakharov[2]
[1]INMED, INSERM UMR1249, Aix-Marseille University, Marseille, France; [2]Kazan Federal University, Kazan, Russia

Background: Research on neuroprotectors in ischemic stroke requires reliable models for screening candidate molecules. Here we explored the neuroprotective effects of several drugs in a model of focal transient cortical ischemia induced by epipial application of the potent vasoconstrictor endothelin-1 (ET-1).
Materials and methods: Head-restrained, urethane-anesthesized Wistar rats of both sexes were used. After craniotomy, 16-channel silicone probes were placed in the somatosensory cortex to record local field potentials and multiple unit activity. ET-1 was applied epipially to the somatosensory cortex during 1 h followed by a 3-h washout. Functional recovery after ET-1–induced ischemia was assessed by the level of recovery of multiunit and field potential activity compared with control values. The size of the ischemic focus was assessed by histological analysis of brain sections. Neuroprotection drugs were applied epipially 1 h before and during ET-1 application.
Results: In control experiments, ET-1–induced ischemic focus was characterized by profound suppression of MUA and cone-shaped lesions in the somatosensory cortex. Neuroprotective effects were investigated for the following drugs: A1 adenosine receptor agonist cyclopentyladenosine (CPA, $n = 18$), antioxidant N-acetylcysteine (NAC, $n = 8$), vasodilators CGRP ($n = 8$) and sodium nitroprusside (SNP, $n = 5$), and the selective NMDA receptor antagonist D(−)-2-amino-5-phosphonopentanoic acid (dAPV, $n = 10$). Among these drugs, CPA had the most pronounced neuroprotective effects, including significant alleviation of functional damage and reduction in the size of histological lesions. NAC and dAPV displayed significant but subtler neuroprotective effects, whereas CGRP and SNP showed no neuroprotection in the ET-1 model.
Conclusions: Neuroprotective screening showed that CPA and to less extent NAC and dAPV were effective neuroprotective agents in the ET-1 transient focal ischemia model.

57ASM – 102 | Dynamics of functional impairments during focal transient ischemia in three-dimensional cortical space

R. Khazipov[1]; D.Vinokurova[2]; A. Zakharov[2]
[1]INMED, Aix-Marseille University, Marseille, France; [2]Kazan Federal University, Kazan, Russia

Background: Development of ischemic injury in cerebral cortex is associated with the depression of electrical cortical activity and the emergence of pathological patterns of spreading depolarizations (SD) and negative ultraslow potentials (NUP). Dynamics of these changes in electrical activity during ischemia in three-dimensional cortical space remain poorly understood.
Materials and methods: We used multielectrode arrays to explore changes in electrical activity across layers of cerebral cortex using two 16-channel silicone probes, one placed into the ischemic focus and another at 2–3 mm distance, 60-channel epidural electrocorticography and optical intrinsic signal imaging during focal ischemia induced by intracortical injection of the potent vasoconstrictor endothelin-1 (ET-1) in the head restrained rats under urethane anaesthesia.
Results: Formation of the ischemic focus after ET-1 administration was associated with clusters of SDs. First, SDs originated from the site of ET1 injection and spread around through all cortical layers. Within the focus, SD initiation progressively shifted to the deep layers, whereas electrical activity did not recover between SDs. Following SDs originated in penumbra, failed invading isoelectric focus and spread around often compartmentalizing to the superficial layers of surrounding cortex. SD-initiated NUPs were most prominent in the electrodes closest to ET-1 injection site, where they attained maximal amplitude by 1 h and waned 3 h after ET-1 injection. Electrographic activity was severely suppressed, particularly in superficial layers, in the focus formed 3 h after ET-1 injection but recovered to the pre-ET1 levels at the distant sites. Spatial characteristics of functional impairments corresponded to the histological lesion in coronal brain sections.
Conclusions: Our findings suggest that the development of cerebral injury during focal ischemia is associated with a formation of ischemic focus expanding in horizontal and vertical cortical dimensions and supported by SDs generated in ischemic penumbra.

57ASM – 107 | Cortical network activity modulation by breathing in juvenile rats during slow-wave sleep

M. Minlebaev[1]; V. Shumkova[2]; V. Silaeva[2]; A. Logashkin[2]; A. Mamleev[2]
[1]*Inmed, Inserm, Aix-marseille University, Marseille, France;* [2]*Kazan Federal University, Kazan, Russia*

Background: Organized neuronal activity underlies brain functioning. Both information processing and transmission between cortical structures occur due to synchronized neuronal activity. The state of deep anesthesia or deep sleep is also characterized by the presence of organized neural activity—slow oscillations (SO). Under these states, the central nervous system is considered to be sensory deprived. While SOs have a high level of synchronization between different cortical areas, little is known about the mechanisms that coordinate SOs during sleep or anesthesia.

Material and Methods: To answer this question, we characterized the phase-lock of cortical activity to breathing during SO. The experiments were done on maturing rats, p19–29. The animals were anesthetized with urethane (1.5 g/kg) intraperitoneally. Cortical activity was recorded using a 16-channel silicon-based electrode. Breath was recorded using a piezoelectric element placed under the animal's chest.

Results: Despite the difference in frequency of SOs and breathing, we found a phase-lock of cortical activity to breathing cycles. Our results showed that the highest probability of periods of cortical activity is observed during the exhalation phase of the anesthetized animal, while the inhalation is accompanied by a decrease in cortical activity.

Conclusions: Our data show that the network activity of the somatosensory cortex is coordinated by breathing in the state of sensory deprivation of the central nervous system. The presented data indicate that breathing provides a constant flow of rhythmic signals to the brain, regardless of the state of the central nervous system. It is possible that this coordination is a necessary condition for the functional integration of local neural ensembles into the general cortical activity under conditions of reduced sensory input.

57ASM – 108 | Effects of dexmedetomidine hydrochloride on sleep–wake cycles and pain sensitivity of newborn rodents in vivo

M. Minlebaev[1]; A. Logashkin[2]; A. Mamleev[2]; V. Silaeva[2]; V. Sitdikova[2]
[1]*Inmed, Inserm, Aix-marseille University, Marseille, France;* [2]*Kazan Federal University, Russia*

Introduction: Urethane is a widely used anesthetic agent for acute in vivo neuroscientific studies. Recently, the restrictions on urethane use appear; therefore, the effective replacement is required. The α-adrenergic receptor agonist dexmedetomidine hydrochloride (Dex) could be a good candidate for that. Commonly it is used in combination with ketamine, its own effects on central nervous system (CNS) functioning remain largely unknown. We have attempted to characterize the effects of Dex on neonatal rat brain functioning.

Materials and methods: To verify Dex, its effect on sleep–wake cycles and pain sensitivity were characterized. Tail flick test was done, and the neck muscle activity was recorded in newborn rats in vivo in control and after injection of Dex (33 μg/kg) or urethane (1 g/kg).

Results: Injection of Dex resulted in slight changes in sleep–wake cycles. Sleep/wakefulness ratio slightly increased (from 1.75 ± 1.86 to 1.85 ± 0.68, $p > .05$), and the active/quiet sleep ratio non significantly decreased (from 2.14 ± 1.57 to 2 ± 0.25). While Dex-treated neonatal rat demonstrated weak difference with the non-treated neonatal rat pup sleep–wake behavior, Dex injection evoked strong analgesic effect. The tail flick reaction time increased up to 1.85 ± 0.42 times (from 1.28 ± 0.26 s in control to 1.9 ± 0.66 s in Dex). Similar analgesic effects were observed in urethane-treated rat pups. In contrast to Dex urethane completely abolished sleep–wake cycling.

Conclusion: We demonstrate that Dex has a slight effect on sleep–wake cycling, but strongly decreases pain sensitivity in newborn rats. Despite the necessity to continue further investigations to characterize the dose dependence, Dex could be considered to be the candidate for urethane replacement for in vivo neurophysiological studies.

57ASM – 109 | Model of focal epileptiform activity induced by intracortical injection of 4-aminopyridine in juvenile rats in vivo

M. Minlebaev[1]; V. Shumkova[2]; V. Silaeva[2]; V. Sitdikova[2]; A. Logashkin[2]; A.Mamleev[2]; D. Suchkov[1]
[1]Inmed, Inserm, Aix-marseille University, Marseille, France; [2]Kazan Federal University, Kazan, Russia

Introduction: Epilepsy is a neurological disorder that affects up to 1.5% of the world population. Understanding the epilepsy's mechanisms is crucial to develop the treatment strategy. In most experimental models of acute epilepsy, the systemic injection of proepileptiogenes is used. Their spread in the nervous tissue could modify the experimental results. Therefore, the model of spatially restricted epileptogene injection is required.

Materials and methods: Focal epileptiform activity was induced by local injection of 200 nL of 4-aminopyridine (4AP) through a carbon tube with an inner diameter of 70 μm into the somatosensory cortex of the juvenile Wistar rats in vivo. To characterize the spread of proepileptogene, the intracellular activity in the current clamp mode was recorded. The half-width changes of the action potentials were characterized before and after the 4AP injection.

Results: A single local 4AP injection (0.2 μL) was followed by multiple episodes of ictal activity. Analysis of the action potentials half-width time showed a difference in neurons located at different distances from the proepileptogene injection site. The neurons recorded at a proximity from 4AP injection (less than 0.7 mm) showed a significant increase in the action potential half-width (1.8 ± 0.4 ms before and 4.2 ± 1.5 ms after 4AP injection). But the duration of action potentials at a distance of more than 1.5 mm from the injections is weakly dependent on the 4AP injection (half-width before injection 1.8 ± 0.4 ms, after—1.9 ± 0.2 ms).

Conclusion: Our results show that local intracortical injection of 4AP is spatially restricted, and 4AP-induced ictal discharges in this case may serve as a model for studies of the epileptiform activity in the intact neuronal tissue.

57ASM – 140 | Effects of acute stress on the CCK+ interneurons and SPW-Rs in the hippocampus

R. Khazipov[1]; A. Vazetdinova[2]; F. Valiullina-Rakhmatullina[2]; R. Miftahov[2]; J. Jappy[3]; A. Rozov[3]
[1]INMED, Aix-Marseille University, Marseille, France; [2]Kazan Federal University, Marseille, Russia; [3]Federal Center of Brain Research and Neurotechnologies, Institute of Fundamental Neurology, Moscow, Russia

Background: Acute stress causes an increase in endocannabinoid tissue concentration, which in turn, through the activation of presynaptic cannabinoid receptors, can selectively inhibit the component of inhibition provided by CCK + INs and thereby change rhythmogenesis in the hippocampus. Although the role of perisomatic inhibition in the generation of sharp wave–ripples (SPW-Rs) is well established, the contribution of CCK + INs has not been sufficiently studied. There is evidence indicating that CCK + INs can be active during the periods between SPW-Rs, determining the duration of intra-SPW-R intervals. We hypothesized that stress-induced inhibition of CCK + IN function may lead to an increase in the frequency of hippocampal SPW-Rs.

Materials and Methods: Acute stress was caused by placing the mouse on a 10 × 10 cm elevated (1 m) platform for 30 min. In the first series of experiments, paired recordings of connected CCK + INs and pyramidal cells were used. In a second series of experiments, SPW-Rs were recorded extracellularly using a 16-shank probe placed along the CA1 pyramidal cell layer.

Results: The application of the CB1 receptor antagonist AM 251 led to an increase in IPSP amplitudes in CCK + IN to pyramidal cell synapses in both control and stressed mice. However, the AM 251–induced increase in the amplitude of IPSPs in slices obtained from stressed mice was significantly larger. Application of AM 251 significantly reduced the frequency of occurrence of SPW-R without affecting their amplitude. These observations are consistent with the hypothesis that the impact of CCK + INs on network activity can change significantly in response to stress.

Conclusions: Acute stress inhibits synaptic strength at CCK + IN to pyramidal cell synapses and modulates the functional contribution of CCK + INs to hippocampal network activity through the endocannabinoid.

57ASM – 143 | Postnatal development of feedforward inhibition in the temporoammonic pathway from entorhinal cortex to CA1 hippocampus

I. Khalilov[1]; A. Gainutdinov[1]; G. Valeyeva[2]; R. Khazipov[1]
[1]*INMED, Aix-Marseille University, Marseille, France;*
[2]*Kazan Federal University, Kazan, Russia*

Background: GABAergic synapses are established prior to glutamatergic synapses in CA1 hippocampus during the neonatal period. However, the development of hippocampal GABAergic circuits associated with specific excitatory pathways remains elusive. Here, we studied developmental changes in the GABAergic inhibition associated with the temporoammonic pathway from entorhinal cortex (EC), which is one of the major excitatory inputs to CA1 hippocampus.
Materials and methods: Electrophysiological recordings were performed from CA1 region in the preparation of intact neonatal mouse hippocampus in vitro. Whole-cell recordings of CA1 pyramidal cells were used to voltage-separate glutamatergic and GABAergic postsynaptic currents (Glu- and GABA-PSCs) evoked by stimulation of EC inputs in the angular bundle.
Results: Starting from postnatal day [P] 1, angular bundle stimulation evoked monosynaptic short-latency Glu-PSCs in CA1 pyramidal cells that coincided with extracellular field excitatory responses in the stratum lacunosum-moleculare, where EC to CA1 synapses are located. Angular bundle-evoked GABA-PSCs were absent in one-third of CA1 neurons during the first postnatal week, and if present, they occurred at long (tens to hundreds of milliseconds) and variable trial-to-trial delays. The window of the temporal integration, defined as the delay of GABA-PSCs from Glu-PSCs in neurons expressing both types of currents, decreased from [Age: median] P1-3: 120 ms to P4-5: 30 ms, P6-7: 7 ms and P8-11: 4 The jitter in GABA-PSC delays from Glu-PSCs showed a similar developmental decrease from P1-3: 50 ms to P8-11: 4 ms.
Conclusions: Thus, feedforward GABAergic inhibition associated with the temporoammonic pathway is very immature during the first postnatal week, supporting an integrative, inhibition-free mode of function in the developing entorhinal-hippocampal system, whereas the switch to fast and reliable feedforward inhibition supporting sparse coding occurs during the second postnatal week.

57ASM – 154 | Developmental changes in the feedforward inhibition in dentate granular cells

I. Khalilov[1]; A. Lukoyanova[2]; L. Safeeva[2]; R. Khazipov[1]
[1]*INMED, Aix-Marseille University, Marseille, France;*
[2]*Kazan Federal University, Kazan, Russia*

Background: Granular cells of dentate gyrus receive main excitatory input from the entorhinal cortex (EC) via the perforant pathway. In adults, the excitatory input from EC to granular cells is rapidly curtailed by feedforward inhibition setting a short (few milliseconds) temporal window for the integration of excitatory inputs and supporting sparse coding. However, interneurons, which are involved in the feedforward inhibition, and their integration in the dentate circuit display delayed postnatal maturation suggesting a delayed development of the feedforward inhibition. Here, we explored the developmental changes in the temporal integration window in the perforant pathway in the mouse dentate gyrus.
Material and methods: We used whole-cell patch-clamp recordings from dentate granular cells in entorhinal-hippocampal slices of postnatal days P1 to P70 mice. Excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were evoked by electrical stimulation of the perforant path and were voltage-separated using low-chloride pipette solution.
Results: Perforant path-evoked responses in granular cells were typically characterized by a sequence of excitatory postsynaptic currents (EPSCs) followed by inhibitory postsynaptic currents (IPSCs) at all ages. Short latency monosynaptic EPSCs could be evoked in granular cells starting from P1-2, and their delay from stimulus did not show variability or age dependence. IPSCs delay was strongly age-dependent, however. During the first postnatal week, IPSC delays attained up to tens of milliseconds and displayed high variability between the responses. Both IPSC delays and their trial-to-trial variability progressively decreased with age to attain adult values by the end of the first postnatal month. As a result, temporal integration window, which was assessed as a difference between EPSC and IPSC delays, was strongly reduced during the postnatal period.
Conclusions: Thus, feedforward inhibition within the entorhinal-dentate perforant pathway undergoes significant changes during postnatal development and enables a large integration window for the excitatory inputs from EC in granular cells during the neonatal period.

57ASM – 156 | Bilateral synchronization of hippocampal theta oscillations in vitro

I. Khalilov[1]; A. Gainutdinov[1]; G. Valeyeva[2]; R. Khazipov[1]
[1]*INMED, Marseille, France;* [2]*Kazan Federal University, Kazan, Russia*

Background: Hippocampal theta oscillations are pivotal for hippocampal functions of spatial navigation, learning and memory. In the behaving animals in vivo, hippocampal oscillations display bilateral synchronization. Internally generated oscillations in the theta frequency range have been also described in the intact hippocampus preparation in vitro. Whether and how theta oscillations are synchronized between the left and right hippocampi in vitro remains elusive.

Materials and methods: We used preparation of the intact hippocampi interconnected by the ventral hippocampal commissure in vitro prepared from juvenile and adult mice. Local field potentials and multiunit activity were recorded using extracellular electrodes from the pyramidal cell layer and stratum radiatum of the left and right hippocampi.

Results: Neuronal network activity in the left and right hippocampi was organized in oscillations within the theta frequency range, which strongly modulated neuronal firing of CA1 neurons. Both neuronal activity and field potential theta oscillations were high levels of bilateral coherence with nearly zero time lag between theta oscillations in the left and right hippocampi. Theta oscillations persisted on both sides, but their bilateral synchronization was eliminated after a surgical cut of the ventral hippocampal commissure.

Conclusions: Theta oscillations are synchronized in the left and right hippocampi in vitro, and their bilateral synchronization is provided by the ventral commissural connections.

57ASM – 165 | Effects of the chemopreventive polyphenol "CURcumin" and its derivative "VANillin" on tumour-associated inflammation and tumour metabolism

Z. El Rashed[1]; U. Pfeffer[1]; S. Ravera[2]
[1]*IRCCS Ospedale Policlinico San Martino, Genova, Italy;* [2]*University of Genova, Department of Experimental Medicine- Human Anatomy, Genova, Italy;* [3]*IRCCS Ospedale Policlinico San Martino, Genova, Italy*

Many studies have reported anti-tumoral activities of the polyphenol CURcumin (CUR). The dual-specificity Tyrosine Phosphorylation Regulated Kinase 2 (DYRK2) and ATP-synthase have been identified as two recent molecular targets of polyphenols. Since DYRK2 is involved in protein degradation regulation via the proteasome, it is implicated in cell proliferation and apoptosis by degrading IkB and consequently determining the nuclear translocation of active NFkB. ATP synthase plays a pivotal role in tumour metabolism, and its inhibition causes a low energy status not always counteracted by the anaerobic glycolysis enhancement. We investigated the effects of CUR and one of its derivatives, vanillin (VAN) on tumour metabolism by examining NF-kB activation and DYRK2 activity using MDA-MB-231 cells as a cellular model, treated or not with polyphenols, in the presence or absence of the synthetic proteasome inhibitor Bortezomib (BRZ) or the IkK inhibitor SC-514. The effect of polyphenols on NFkB-related genes was tested by evaluating NA and protein expression. Nuclear localization and phosphorylation of NFkB subunits were assessed by enzyme-linked immunosorbent assay. The effect of polyphenols on cellular metabolism was monitored by testing ATP synthase activity, ATP/AMP ratio, and lactate dehydrogenase enzyme activity. Our results show that both polyphenols significantly reduce tumour-associated inflammation by downregulating the expression of NFkB-related genes, a slow down the tumour metabolism by inhibiting ATP synthesis. The combination of SC-514 with CUR and VAN did not lead to any additive effect as compared to cells treated with polyphenols treatment alone, suggesting that the CUR and VAN effects on ATP synthase inhibition are independent of NFκB inhibition. However, polyphenols showed a significant additional inhibitory effect on ATP synthesis in the presence of the proteasome inhibitor BRZ. In conclusion, CUR and VAN anti-tumoral effects were investigated by their potential to inhibit cell proliferation by targeting tumour inflammation and energy metabolism.

SYMPOSIUM 13 – OTHER TOPIC CLINICAL RESEARCH

57ASM – 008 | Correlation of MYD88 and TNFAIP3 mutations with clinico-haematological profile in Diffuse Large B cell lymphoma

R. Shaat[1]; H. A. Abd El Ghaffar[1]; S. Shamaa[2]; H. M. Abdel-masseih[1]; O. Elbaz[1]
[1]clinical pathology department Mansoura University-faculty of medecine, Mansoura, Egypt; [2]clinical oncology department Mansoura University-faculty of medecine, Mansoura, Egypt

Background: Activated B-cell–like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL) is characterized by chronic active B-cell receptor signalling and a constitutive activation of the nuclear factor kappa B (NF-KB) pathway. As a driving force of NF-KB overactivity, myeloid differentiation primary response gene 88 (MYD88) L265P mutation occurs in ABC-DLBCL. However, the MYD88 L265P mutation has not yet been studied with tumour necrosis factor alpha induced protein3 (TNFAIP3) mutation in DLBCL cases.

Aim: to determine the prevalence of MYD88 and TNFAIP3 mutations and their relationship with the clinico-haematological profile in DLBCL.

Methods: we investigated 100 DLBCL patients for MYD88 L265P and TNFAIP3 mutation by real time polymerase chain reaction.

Results: MYD88 L265P CT heterozygous genotype was found in 20% of cases. CT heterozygous genotype was significantly frequent in ABC type, stage IV, higher IPI groups, extra-nodal infiltration and BM infiltration. It was also associated with shorter overall survival (OS). TNFAIP3 mutation GA heterozygous genotype was found in 18% of the patients where 77.8% of them were ABC-DLBCL subtype. GA heterozygous genotype was frequently associated with stage IV, and extra-nodal infiltration and shorter OS.

Conclusion: Our study suggests a role of MYD88 mutation in the pathogenesis and prognosis of DLBCL.

57ASM – 010 | Biochemical identification of cysteines involved in tumour-specific conformation of the ovarian cancer marker NaPi2b

D. Reshetnikova[1]; L. Bulatova[1]; A. Kilunov[1]; A. Korotaeva[1]; V. Skripova[1]; D. Savenkova[1]; A. Nurgalieva[1]; R. Kiyamova[1]; M. Bogdanov[2]
[1]Biomarker Research Laboratory, Kazan Federal University, Kazan, Russian Federation; [2]Department of Biochemistry and Molecular Biology, University of Texas Health Science Center, Houston, USA

Background: The sodium-dependent phosphate transporter NaPi2b is a membrane protein overexpressing in ovarian tumours and is considered as a promising target for ovarian cancer therapy. Despite the presence of the NaPi2b in normal cells, the therapeutic monoclonal antibodies targeting the large extracellular domain (ECD) of NaPi2b accumulate predominantly in tumour cells. The ECD contains 4 cysteines potentially forming disulfide bonds with each other to provide potential tumour-specific conformation of the large extracellular domain of NaPi2b. The number of disulfide bonds maintaining the potential tumour-specific conformation of the ECD of NaPi2b remains unknown. Hence, the aim of this work is to prove the number of disulfide bonds formed in the ECD is crucial for antibody recognition. This information might be the basis for studying the mechanism of tumour-specific ECD conformation for developing more effective anticancer target therapy.

Methods: Intact ovarian cancer cells OVCAR-4 expressing NaPi2b were pretreated with or without reducing agent TCEP with the following washing and treating with mPEG-Mal, which covalently modifies free thiol groups, giving 5 kDa to each. The number of modified cysteine thiol groups was determined by the shift of the specific band corresponding to NaPi2b in Western blot by antibodies recognizing an N-term of NaP2b.

Results: Pretreatment of the intact OVCAR-4 cells with TCEP followed by mPEG-Mal led to increasing the molecular weight of NaPi2b by approximately 20 kDa corresponding to 2 disulfide bonds within the ECD. The mobility of NaPi2b treated only with mPEG-Mal did not change, suggesting the absence of free cysteine thiol groups within the ECD.

Conclusions: Our results supported by the Kazan Federal University Strategic Academic Leadership Programme (PRIORITY-2030) have shown that all 4 cysteines of ECD are involved in the formation of the two disulfide bonds crucial for the potential tumour-specific conformation of the NaPi2b transporter ECD.

57ASM – 012 | Transmembrane distribution of phosphatidylethanolamine in plasma membrane of ovarian cancer cells under conditions mimicking tumour microenvironment

D. Savenkova[1]; L. Bulatova[1]; I. Makarenko[1]; D.Reshetnikova[1]; V. Skripova[1]; R. Kiyamova[1]; M. Bogdanov[2]

[1]*Biomarker Research Laboratory, Kazan Federal University, Kazan, Russian Federation;* [2]*Department of Biochemistry and Molecular Biology, University of Texas Health Science Center, Houston, USA*

Background: In healthy cells rather than being evenly distributed phosphatidylserine (PS) and phosphatidylethanolamine (PE) are found preferentially in the inner leaflet of the plasma membrane. Unlike normal cells, tumour cells lose the ability to maintain aminophospholipid asymmetry and expose PS naturally which promotes tumour growth and immune suppression by not established mechanisms. Since both PS and PE are co-regulated by the same transporters in the case of PE translocation it also might serve as a specific lipid biomarker diagnostic and prognostic value of which is yet to be determined.

Aim: We aim to investigate whether hypoxia and low pH characteristics of tumour microenvironment (TME) might promote PE translocation on the surface leaflet of cancer cell membranes.

Methods: Ovarian cancer cells OVCAR-4 were incubated under standard (pH = 7.5, O2 = 21%) and TME modelling conditions (pH = 5.8, O2 = 1%) following with a two-step staining with PE-specific biotinylated Ro09 and streptavidin conjugated with Alexa Fluor 405. Propidium iodide (PI) staining was conducted to justify plasma membrane integrity. Visualization of PE on living OVCAR-4 cells was performed with the use of laser confocal microscopy.

Results: Under normoxic conditions signal from PE was not detected on the surface of intact OVCAR-4 cells. However, under TME conditions cells were positive for PE staining, but not for PI, demonstrating that PE is translocated from the inner to the outer leaflet of cancer cell membrane.

Conclusions: Our results supported by the Kazan Federal University Strategic Academic Leadership Programme (PRIORITY-2030) provided nonapoptotic externalization of aminophospholipids in cancer cells have become a subject of great interest due to correlation with innate immune suppression and promotion of tumour growth. We propose that TME-driven surface exposure of PE, a second abundant phospholipid in cell membrane, coincides with constitutive PS externalization and can serve as TME specific potential therapeutic target and/or diagnostic tool for ovarian and other types of cancer

57ASM – 125 | The role of phospholipase A2 from pathogenesis to treatment of familial Mediterranean fever

H. Sargsyan; Petros Ghazaryan

Yerevan State Medical University, Arabkir JMC, Yerevan, Armenia

Familial Mediterranean fever (FMF) is an autosomal recessive auto inflammatory disease is expressed by acute spells of fever, painful manifestation in the abdomen, chest and joints. Sometimes, splenomegaly is included in the clinical signs. Due to the importance of phospholipase A2 (PLA2) in inflammatory responses, as well as according the literature data and the results of the work done by the former of our studies we attempt to identify the role of PLA2 in different clinical signs of FMF children.

The aim of our study is to investigate change of activity of PLA2 with non complicated of amyloidosis FMF patients with different clinical signs before and after colchicine therapy.

Clinical studies conducted in 61 non complicated of amyloidosis FMF children "Arabkir" JMC. The age of the patients varies from 10–15. We are selected as a control group of 27 healthy people in practice. Determination of the activity of PLA2 was conducted in erythrocytes membrane by spectrophotometric method in Haematological Center of Armenia. The doses of colchicine therapy – 1.0-1.5 mg/day.

The results of activity of PLA2 before/after colchicine therapy in different clinical signs are following: thoracic forms – $21.91 \pm 2.28/18.95 \pm 5.19$, $p > 0.05$; abdominal forms- $28.57 \pm 1.70/20.97 \pm 2.03$, $p < 0.01$; thoraco-abdominal forms – $35.70 \pm 1.28/23.20 \pm 0.91$, $p < 0.001$, FMF with arthropathy – $28.25 \pm 1.93/26.78 \pm 1.53$, $p > 0.05$; FMF with splenomegaly $33.33 \pm 0.96/30.69 \pm 2.73$, $p < 0.05$: The activity of PLA2 in control group was 15.18 ± 1.03. Our results showed significant suppression of the activity of PLA2 after taking colchicine by patients of FMF. Should be noted, that despite the therapy the activity of PLA2 under arthropathy and splenomegaly remained still high. The results in our experience are established the important role of PLA2 in pathogenesis of FMF, as well as the significant role in the process of treating the disease. Hence, It is an informative test for the evaluation of the efficiency of colchicine therapy of FMF patients.

57ASM – 130 | The efficacy of complement inhibitors in the treatment of paroxysmal nocturnal hemoglobinuria: A systematic review and network meta-analysis

A. Elrosasy[1]; A. Ramadan[2]; B. Elsayed[3]; A. Zabady[4]; M. Alaa[5]; O. Altobaishat[6]; M. Tanashat[7]; A. Elayan[8]; N. Abouabed[9]; Y. Soliman[10]
[1]*Faculty Of Medicine, Cairo University, Cairo, Egypt;* [2]*South Valley University, Faculty of Medicine, Qena, Egypt, Qena, Egypt;* [3]*Faculty of Medicine Ain Shams University, Cairo, Egypt, Cairo, Egypt;* [4]*Faculty of science Damanhour University, Buheira, Egypt, Damanhour, Egypt;* [5]*Faculty of Medicine Fayoum University, Fayoum, Egypt;* [6]*Faculty of medicine, Jordan University of science and technology, Amman, Jordan;* [7]*Faculty of medicine, yarmouk University, Irbid-jordan, Yarmouk, Jordan;* [8]*Faculty of Pharmacy, Philadelphia University, Amman Jordan, Amman, Jordan;* [9]*Faculty of Medicine, Setif, Algeria, Setif, Algeria;* [10]*Faculty of Medicine, Assiut University, Assiut, Egypt*

Background: Due to understanding the role played by the complement system in causing paroxysmal nocturnal hemoglobinuria (PNH), researchers have looked into the potential of complement inhibition as a therapeutic strategy for managing this disease. PNH is a life-threatening disorder of the blood, as the disease is marked by the breakdown of red blood cells (RBCs), blood clotting, and compromised bone marrow function. The aim of this network meta-analysis was to compare the efficacy of the different drugs used in the treatment of PNH.
Materials and Methods: Following the PRISMA guidelines and the Cochrane risk-of-bias tool (RoB 2), six randomized controlled trials (RCTs) were included in the present network meta-analysis with a total of 781 patients after a comprehensive literature search for RCTs. We used the established analytical tool (MetaInsight V4.0.0) and data extraction sheets to analyse the outcome data. The main outcome examined was the lactate dehydrogenase (LDH) level which reflects the hemolysis of RBCs.
Results: Compared to the placebo, subcutaneous ravulizumab had the highest efficacy in reducing LDH levels (U/L) (Mean difference (MD) = −2128.52 with 95% confidence interval (CI) [−2662.67; −1594.38]), followed by pegcetacoplan (MD = −2146.90 with 95% CI [−2708.46; −1585.34]), intravenous ravulizumab (MD = −2097.42 with 95% CI [−2628.18; −1566.66]), eculizumab biosimilar (MD = −1930.80 with 95% CI [−2767.25; −1094.35]), and eculizumab (MD = −2034.00 with 95% CI [−2564.32; −1503.68]).
Conclusion: Our analysis showed that subcutaneous ravulizumab had the most significant efficacy in reducing RBCs hemolysis that was indicated by LDH levels, while eculizumab had the lowest efficacy; however, further RCTs need to be conducted to confirm these results, as a limited number of patients have been investigated in the conducted RCTs.

57ASM – 136 | AR-V7 splice variant detection and its value as prognostic biomarker in metastatic castration-resistant prostate cancer

A. J. Guerra[1]; E. Tavares Silva[1]; M. Eliseu[1]; M. Coucelo[2]; A. M. Ferreira[1]; A. T. Simões[2]; B. Parada[1]; A. do Figueiredo[1]
[1]*Serviço de Urologia, CHUC, Coimbra, Portugal;* [2]*Unidade Funcional de Hematologia Molecular, CHUC, Coimbra, Portugal*

Background: Treatment of castration-resistant prostate (mCRPC) is becoming more targeted, with the rising of new prognostic biomarkers, such as ARV7, a splice variant that encodes an isoform of androgen receptor (AR), for seeming to be a predictive factor of worse response to new hormonal agents.
Objectives: To describe the population of men with mCRPC who were surveyed for the presence of ARV7 in relation to therapeutic outcomes, regarding new hormonal agents; to assess ARV7 potential prognostic value.
Methods: We conducted a cross-sectional study including all patients with mCRPC, treated in a tertiary hospital center, between 01/01/2017 and 01/01/2022, who underwent AR-V7 analysis. CTC were isolated from whole blood using AdnaTest ProstateCancerSelect (Qiagen), NA extracted, and RT-qPCR was used for AR-V7 detection using b-Actin. For survival analysis, Kaplan–Meier estimate was used.
Results: We identified a population of 15 patients. ARV7 was positive (AP) for 6 patients and negative (AN) for 9. OS after ARV7 sampling was 30.2 ± 18.6 months in the AN group and 6.7 ± 4.7 months in the AP, with no statistical difference ($p = 0.507$). However, OS after ARV7 sampling was statistically bigger in the AN group ($p < 0.001$). The mean time to progression from mHSPC to m-CRPC showed no statistical difference between both groups ($p = 0.89$). In the mCRPC stage, the mean time after starting new hormonal agents until progression and beginning of a new line of treatment or death was 16.3 ± 8.6 months in the AN group and 9.7 ± 6.3 months in the AP group, despite showing no statistically significant difference ($p = 0.096$).
Discussion: There is a tendency for worse response to treatment with new hormonal agents and overall survival in the ARV7 positive group, which reinforces the

importance of this biomarker in therapeutic decision-making and prognostic tool in patients with mCRPC. Further studies with bigger samples will be necessary to determine its clinical applicability.

57ASM – 137 | Role of BNP and NT-proBNP for early diagnosis of cardiac pathologies in pregnancy

M. Uzakova[1]; G. Babajanova[1]; J. Uzokov[2]
[1]*Tashkent Medical Academy, Tashkent, Uzbekistan;* [2]*Republican specialized scientific practical medical center of therapy and medical rehabilitation, Tashkent, Uzbekistan*

Introduction and Background: Brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide considered one of the biomarkers for the early diagnosis of cardiac pathologies. Role of these biomarkers in pregnancy with cardiac pathologies have not been fully studied. Aim of the study was to evaluate the possible prognostic value of BNP and NT-proBNP for the early diagnosis of cardiac pathologies in pregnancy.

Material and Methods: 118 pregnant women with cardiac pathologies and 32 pregnant women without any cardiac pathologies (control group) have been enrolled in the study (I and II trimester). Among group I pregnant women 32 had arrhythmias, 26 had myocarditis and 60 had hypertension. Baseline anthropometric, clinical and laboratory data along with assessing the BNP and NT-proBNP were performed. All statistical analysis were conducted using SPSS 26.01 (USA).

Results: Baseline anthropometric data were similar in each group ($p > 0.05$). Among clinical parameters dyspnea and fatigue were more pronounced in group I than group II pregnant women ($p < 0.05$). BNP were significantly higher in group I than Group II (178.74 ± 56.45 pg/mL vs. 83.12 ± 24.6 pg/mL, $p < 0.05$). When we separately analysed by cardiac pathologies, BNP was significantly higher in myocarditis followed by hypertension and arrhythmias (195.24 ± 46.76 pg/mL vs. 174.12 ± 54.28 pg/mL vs. 148.12 ± 63.25 pg/mL, $p < 0.05$). As far as NT-proBNP was concerned, group I tended to have higher level of this biomarker than group II (254.67 ± 82.12 vs. 156.28 ± 65.35 pg/mL, $p < 0.001$). Among cardiac pathologies, myocarditis had higher level of NT-proBNP followed by hypertension and arrhythmias (285.27 ± 75.12 vs. 251.36 ± 79.12 vs. 231.28 ± 95.18 pg/mL, $p < 0.05$). When we analysed correlation BMI and biomarkers, there were positive correlation between them in Group II but not in Group I ($p < 0.05$).

Conclusions and Recommendations: BNP and NT-proBNP significantly increased in pregnant women with cardiac pathologies. Early screening of the biomarkers might be essential tool for the early diagnosis of cardiac pathologies in pregnancy.

57ASM – 190 | BIO-Ra score as predictor of prognosis in castration resistant metastatic prostate cancer receiving Radium-233

A. M. Ferreira[1]; J. Lorigo[1]; A. J. Guerra[1]; I. Marques[3]; I. Casimiro[2]; E. Tavares da Silva[1]; G. Costa[2]; P. Nunes[1]; A. Figueiredo[1]
[1]*Department of Urology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal;* [2]*Department of Nuclear Medicine, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal;* [3]*Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Coimbra, Portugal*

Introduction: Radium-223 is a therapeutic option in metastatic castration-resistant prostate (mCPRC) cancer patients with bone metastases. However, many studies showed that the benefit is lower than that reported in trial clinical trials, probably due to a suboptimal selection of patients. Therefore, the identification of prognostic factors to select mCRPC patients most likely to benefit from this treatment is needed. The BIO-Ra score, which combines clinical factors and peripheral inflammatory indices in a multifactorial score, is in study as a tool for helping patient's selection for Radium-223 treatment.

Objective: We studied the correlation between the BIO-Ra score and treatment completion with Ra-223 in the patients treated in our institution.

Materials and Methods: We conducted a cross-sectional study of all patients that were treat with at least one cycle of Ra-223 treatment. Clinical data for the BIO-Ra score calculation were collected before the first cycle. All data were statistically analysed.

Results: 31 patients with mCPRC received treatment with Ra-223 in our center. The median overall survival (mOS) of the entire cohort was 15.39 months. Regardind the Bio-Ra Score, the low-risk group had 8 (26.7%) patients, the intermediate-risk group 6 (20%) patients and the high-risk group 16 (53.3%) patients.

Bio-Ra Score showed an inverse correlation with the mOS ($r = -0.41$; $p = 0.034$). In the low-risk group patients completed in median 5.63 cycles, in intermediate risk 5.5 and in high risk group 4.25 cycles ($p = 0.035$).

Conclusion: According to this study, BIO-Ra score correlates with OS and a higher score is associated with lower mOS. Also, a higher score correlates with completing less cycles of the target 6 intended for Ra-223 treatment. This study supports clinical use of Ra-223 score.

57ASM – 209 | Quality of life in patients with bronchial asthma on the background of standard therapy

D. Rakhimova; A. Alyavi
Republican Specialized Scientific Practical Medical Center of Therapy and Medical Rehabilitation, Tashkent, Uzbekistan

Introduction: To study parameters of the quality of life (QL) in patients with bronchial asthma (BA) complicated by cor pulmonale (CP).
Material and Methods: At screening-questioning on the Seattle questionnaire were 32 patients. The control group (CG) was made by 30 healthy volunteers. Depending on the level of average pulmonary arterial pressure and the presence of structurally functional changes of right ventriculi (RV) of heart. All patients were distributed on 2 groups: 1st group – 15 patients with a pulmonary hypertension (PH) and 2nd group – 12 patients with dilatation right ventriculi of heart (DRV).
Results: It was established, that parameters of QL were lowered at all patients with CP in comparison with CG. However, expression of changes in the specified groups is not unequivocal. So, 2nd sick group was worse adapted for moderate physical activity, and among them is sharp restriction of physical activity. More often difficulties were authentically observed at walk, that among patients with PH were observed authentically less often ($p < 0.05$) accordingly. At patients with DRV emotional distress more expressed by low points of an estimation of an emotional condition, in comparison with patients with PH is established. Patients with DRV authentically had fear of physical activity, than patients with PH ($p < 0.03$) is more often.
Conclusion: At patients with DRV the QL degree becomes more perceptible than expressed depression of quality of a life on an emotional condition and professional suitability and satisfaction treatment that it is necessary to consider at carrying out of rehabilitational actions.

57ASM – 213 | Apert Syndrome: Not difficult to diagnose by a single specialist, but multidisciplinary approach is needed for follow-up

L. Caba; M.-C. Panzaru; C. Rusu; E. Vlad Gorduza
University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania

Background: Apert syndrome is a monogenic condition caused by mutations in the FGFR2 gene and characterized by craniosynostosis, midface retrusion, syndactyly in the hands and feet. In some cases associates intellectual disability, heart abnormalities, genitourinary and gastrointestinal tract abnormalities. Apert syndrome has autosomal dominant inheritance.
Materials and Methods: We present the case of a boy clinically diagnosed at birth with Apert syndrome (craniofacial dysmorphia determined by craniosynostosis, syndactyly of fingers 1–5 bilaterally on the hands and feet, nail abnormalities). The boy was clinically re-evaluated at 15 years and 11 months, when neuropsychiatric, ENT examinations, and molecular testing with a panel of 65 genes involved in craniosynostosis were performed.
Results: The clinical examination at the age of 15 years and 11 months highlighted: normal height and weight; craniofacial dysmorphia with turricephaly, midface retrusion, downslanting palpebral fissures, exophthalmos, gingival hypertrophy, dental anomalies, bilateral syndactyly in the hands and feet. Psychomotor development was delayed. The personal history highlights surgical interventions on the hands bilaterally for the correction of syndactyly, nocturnal enuresis (improved under treatment). Family history is negative for Apert syndrome. Neuropsychiatric examination shows average mental retardation QI = 40, nocturnal enuresis. ENT examination highlights sensorineural hypoacusis, medium bilateral form. Molecular testing showed a pathogenic heterozygous variant in the FGFR2 gene: c.758C > G (p.Pro253Arg). This mutation together with the clinical appearance (craniosynostosis, syndactyly in the hands and feet) confirms the diagnosis of Apert Syndrome. The patient's multidisciplinary approach includes multiple medical and surgical specialties and must be done regularly for 6 months – 1 year for a continuous improvement of the quality of life.
Conclusions: We present a case of Apert syndrome in order to highlight the importance of multidisciplinary approach and the molecular testing in allelic disorders and nonallelic disorders differential diagnosis of this rare disease.

SYMPOSIUM – 14 – OTHER TOPIC – COVID-19

57ASM – 050 | Short- and long-term mortality in elderly patients hospitalized for COVID-19: A retrospective analysis investigating prognostic factors

F. Rotunno[1]; B. Pennella[2]; M. Mercuri[1]; F. Troian[3]; C. Berton[3]; M. Guerci[4]; M. Biancucci[5]; M. Molteni[4]; D. Dalla Gasperina[6]; F. Dentali[6]; A. Bonaventura[2]; A. M. Maresca[6]
[1]Ospedale Carlo Ondoli, ASST Sette Laghi, Angera, Italy; [2]Ospedale di Circolo e Fondazione Macchi, ASST Sette Laghi, Varese, Italy; [3]University of Insubria, Varese, Italy; [4]Ospedale Galmarini, ASST Sette Laghi, Tradate, Italy; [5]Ospedale Causa Pia Luvini, ASST Sette Laghi, Cittiglio, Italy; [6]Department of Medicine and Sugery, Insubria University, Varese, Italy

Introduction and Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has posed a high mortality among the elderly. We aimed at identifying potential risk factors associated with mortality in ultra-octogenarian patients hospitalized with coronavirus disease 2019 (COVID-19) in 2020.
Materials and Methods: We retrospectively analyzed data from COVID-19 patients aged ≥80 years hospitalized in Varese and Tradate hospitals (ASST Sette Laghi, Varese, Italy) between 10 October 2020 to 4 May 2021. Clinical information was recorded through electronical medical records. Frailty was assessed using the Clinical Frailty Scale (CFS).
Results: 509 patients were included. Median age was 86 years, with a prevalence of females (59.7%) and of ≥3 comorbidities (47.3%). A large majority of patients (70%) were classified as frail (CFS >4). Most patients presented with respiratory failure, needing high fractions of FiO2 (40%). Overall in-hospital mortality was 39.7% and independent risk factors for this included age, CKD, non-rebreather-mask and CPAP at admission, non-rebreather-mask and CPAP as maximum oxygen support (adjusted hazard ratio [aHR] between 1.08 and 3.89, $p < 0.005$ for all). At 6-month follow-up, overall mortality was 57.8%. Predictors of 6-month mortality were age (aHR 1.08, 95% CI 1.05–1.10), CKD (aHR 1.40, 95% CI 1.05–1.87), dementia (aHR 1.63, 95% CI 1.28–2.07) and chronic liver disease (aHR 1.74, 95% CI 0.97–3.12).
Conclusions and Recommendations: The elderly has been disproportionately affected by COVID-19. Age and comorbidities provide more relevant prognostic information rather than CFS in order to guide clinical decisions.

57ASM – 149 | The prognostic value of lactate-dehydrogenase/albumin-urea and platelet to lymphocyte ratio in the prediction of COVID-19 induced target organ damage

H. Shokr[1]; M. Marwah[2]; H. Siddiqi[3]; F. Wandroo[3]; S. Marwah[3]
[1]The University of Manchester, Pharmacy School, Faculty of Biology, Medicine and Health, Manchester, The United Kingdom; [2]Aston University, Aston Medical School, Birmingham, The United Kingdom; [3]Haematology Department, Sandwell and West Birmingham Hospitals NHS Trust, Birmingham, The United Kingdom

Background: The need for COVID-19 infection prognostic predictors is of paramount interest to optimize clinical care provided to the patients. Various predictive ratios were tested as a potential tool for risk stratification of hospitalized patients. Of these ratios, platelet-to-lymphocyte ratio (PLR) has emerged as a possible prognostic factor especially in critically ill patients, however, evidence supporting its association with morbidity and mortality is weak. Therefore, in this research we assessed the clinical value of PLR compared to a novel COVID-19 prognostic ratio (lactate dehydrogenase/albumin to-urea, LAU) [1],[2],[3] in the prediction of complications associated with COVID-19 infection.
Methods: This study involved blood analyses of 1139 hospitalised COVID-19 survivors and 349 deceased cases post-COVID-19 infection. Laboratory tests included complete blood picture, inflammatory markers and routine organ function tests.
Results: Nonsurvivor group exhibited lower serum haemoglobin and platelet concentrations ($p < 0.0001$) and higher mean corpuscular volume, neutrophil, PLR and LAU compared to individuals who survived the infection ($p < 0.001$, $p < 0.0013$, $p < 0.001$, $p < 0.0126$). Data analysis revealed higher infection-related clinical complications biomarkers in the nonsurvivor group including INR ($p = 0.003$), Urea ($p = 0.029$), C-reactive protein ($p = 0.0001$), alkaline phosphatase ($p = 0.003$), total bilirubin ($p = 0.005$), ferritin ($p = 0.021$) and serum creatinine ($p = 0.0001$). PLR had an area under receiver operating characteristic (ROC) of 0.59 compared to a ROC value of 0.67 using the LAU ratio. Moreover, LAU was significantly correlated to the infection prognostic parameters such as serum urea ($r = 0.424$), ferritin ($r = 0.385$), creatinine ($r = 0.365$), CRP ($r = 0.268$) and D-dimer ($r = 0.176$), ($p \leq 0.05$ in all).
Conclusion: LAU ratio has a strong clinical predictive value of COVID-19 clinical complications. Early assessment of LAU could enable a risk stratification of high-risk patients which could enable early intensive intervention to improve their prognosis.

57ASM – 168 | The cancer incidence during COVID-19 pandemic in a secondary health care clinic from Romania

L. Ciumarnean; M. V. Milaciu; S. C. Vesa
"Iuliu Haţieganu" University Of Medicine And Pharmacy Cluj-napoca, Cluj-napoca, Romania

Introduction: The aim of our study was to compare the total number of hospital admissions, the diagnosis at admission and discharge, in a secondary non-COVID healthcare clinic in Romania, in order to evaluate the cancer incidence in the 2020 COVID-19 pandemic comparative to the same timeframe of 2019.

Material and methods: The study was conducted in the CF Clinical Hospital, Cluj-Napoca, Romania. It compared the COVID-19 pandemic period from 1st of March 2020 to 31 December 2020 to the same timeline of 2019 (1st of March 2019 – 31 December 2019). The study followed the guideline for reporting observational studies STROBE (Strengthening the Reporting of Observational Studies in Epidemiology).

For each timeframe, we recorded the total number of hospital admissions (in the Internal Medicine, Surgery and ENT Departments), the primary diagnoses at admission/discharge, the cancer diagnosis at discharge and if they already had a known diagnosis of cancer.

We collected all the information existing in the hospital database, and there were no exclusion criteria, to present most accurately the actual situation in the hospital. Each patient diagnosed with cancer was counted once, based on the first entry of the respective ICD-10 diagnosis.

Results: In the specified timeframe from 2019, there were 7704 patients admitted to the CF Clinical Hospital, of whom 1232 (15.9%) had a cancer diagnosis. In the specified timeframe from 2020, there were 1776 patients admitted to the CF Clinical Hospital, of whom 344 (19.4%) had a cancer diagnosis. The difference in prevalence was statistically significant ($p < 0.001$).

Conclusion: There was an increased prevalence of cancer cases in the first year of the COVID-19 pandemic.

57ASM – 212 | Influence of angiotensin receptor-neprilysin inhibitors in biochemical markers patients with chronic heart failure after COVID-19

N. Guzal; M. Mukhamedova
Center for the development of professional qualification of medical workers, Tashkent, Uzbekistan

Introduction: Purpose of the study was to assess the influence of angiotensin receptor-neprilysin inhibitors—sacubitril/valsartan in biochemical markers and lipid parameters in patients with chronic heart failure who have undergone COVID-19.

Material and Methods: 140 patients aged 53–78 years (mean age 66.0±16.4 years, male=46%) with CHF who underwent COVID-19 from 2020 to 2022 years were enrolled in the study at the outpatient clinic.

Patients were divided into two groups by 70 according to the severity of the COVID-19. First group patients who have undergone mild to moderate COVID-19 whereas second group patients who have undergone severe COVID-19 within the last 6 months of the examination. All patients were assigned sacubitril/valsartan with the dose of 24/26 mg followed by titrating the dose up to 48/51 mg in addition to the standard treatment during the six months. All laboratory, instrumental and biochemical data were obtained at baseline and after the six months of the treatment.

Results: Initial baseline anthropometric characteristics of patients were similar in 2 groups ($p > 0.05$). On the background of sacubitril/valsartan, N terminal pro B natriuretic peptide (NT-proBNP) has been decreased in both groups during the treatment (from 218.62±51.75 pg/mL to 169.75±42.35 pg/mL in Group I, $p < 0.05$; from 312.35±66.45 pg/mL to 249.72±53.24 pg/mL in Group II, $p < 0.05$), however, they were not observed any statistically significant changes when we compared two groups ($p > 0.05$). Regarding the brain natriuretic peptide (BNP), sacubitril/valsartan tended to have more pronounced effect in patients with CHF who had undergone severe COVID-19 than those mild to moderate COVID-19 (from 96.86±29.52 pg/mL to 85.48±27.32 pg/mL vs. from 167.02±34.18 pg/mL to 101.12±28.48 pg/mL, $p < 0.05$).

Conclusion: Angiotensin receptor-neprilysin inhibitors—sacubitril/valsartan had more positive impact on NT-proBNP, BNP and some lipid parameters in patients with CHF after COVID-19.