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ABSTRACT

POSTERS

P001-T | Disruption of IDH2 attenuates LPS-induced inflammation and lung injury

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Acute lung injury (ALI) is an acute failure of the respiratory system with unacceptably high mortality, for which effective treatment is urgently necessary. Infiltrations by immune cells, such as leukocytes and macrophages, are responsible for the inflammatory response in ALI, which is characterized by excessive production of pro-inflammatory mediators in lung tissues exposed to various pathogen-associated molecules such as lipopolysaccharide (LPS) from microbial organisms. Alpha-Ketoglutarate (alpha-KG) is a key metabolic intermediate and acts as a pro-inflammatory metabolite, which is responsible for LPS-induced proinflammatory cytokine production through NF-kappaB signaling pathway. Mitochondrial NADP+-dependent isocitrate dehydrogenase (IDH2) has been reported as an essential enzyme catalyzing the conversion of isocitrate to alpha-KG with concurrent production of NAPDH. Therefore, we evaluated the role of IDH2 in LPS-induced ALI using IDH2-deficient mice. We observed that LPS-induced inflammation and lung injury is attenuated in IDH2-deficient mice, leading to a lengthened life span of the mice. Our results also suggest that IDH2 disruption suppresses LPS-induced proinflammatory cytokine production, resulting from an inhibition of the NF-kappaB signaling axis in an alpha-KG-dependent manner. In conclusion, disruption of IDH2 leads to a decrease in alpha-KG levels, and the activation of NF-kappaB in response to LPS is attenuated by reduction of alpha-KG levels, which eventually reduces the inflammatory response in the lung during LPS-induced ALI. The present study supports the rationale for targeting IDH2 as an important therapeutic strategy for the treatment of systemic inflammatory response syndromes, particularly ALI.

P002-T | Effects of decrease in intracellular Ca^{2+} concentration on electrical characteristics of premotor interneurons of training snails

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The Ca^{2+} plays an important role in formation of conditioning. The intracellular Ca^{2+}, which is stored in the endoplasmic reticulum and mitochondria, is involved in the regulation of many intracellular reactions. The changes of the level of intracellular calcium concentration by the influx and uptake transport from the endoplasmic reticulum and mitochondria can regulate short-term and long-term forms of plasticity. In present study we analyzed the influence of a decrease in the intracellular Ca^{2+} concentration on the maintenance of the excitable changes of snails premotor interneurons after learning.

It was found that after decreasing of intracellular Ca^{2+} level by the calcium chelators EGTA the threshold potential significantly increased by 3.3 mV in the group of untrained snails and tended to increase, but not significantly in a group of trained snails. Application of membrane-penetrating calcium ion chelator BAPTA-AM did not lead to specific changes of electrical characteristics of trained snails. This difference in actions of both chelators pointed on different pathways of influence of intracellullar Ca^{2+} concentration on neuron excitability. Our results suggested that the decrease of intracellullar Ca^{2+} level was not involved in maintenance of the excitability of interneurons after training. As we showed earlier the changes of intracellullar Ca^{2+} level was more critical on the stage of formation of the conditioning.

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P004-T | Dietary nitrite extends lifespan in the fruit fly

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Aging has been associated with a progressive decrease in nitric oxide (NO) availability due to a diminished endothelial nitric oxide synthase expression and activity and increased oxidative stress resulting in NO degradation. Dietary inorganic nitrate and nitrite are alternative substrates for NO synthase-independent NO generation and stimulation of this pathway has been shown to reduce oxidative stress and enhance mitochondrial efficiency. This makes nitrate and nitrite therapy an appealing nutrition-based intervention to address age-related disorders. The aim of this study was to assess whether dietary nitrite extends lifespan in the fruit fly Drosophila melanogaster and investigate the possible role of mitochondria in any such effects.

In a survival assay, female flies (n = 200/treatment) received a nitrite (0, 0.1, 1, 10 and 100 μmol/L) supplemented media (sugar-yeast 15%) and dead flies were scored three times a week throughout their entire lifespan. In order to study the possible link between mitochondrial respiration and the extended longevity, female flies were fed a 1 μmol/L nitrite-supplemented media for 30 days. The whole fly thorax (n = 8/treatment) was then dissected and mitochondrial respiration was measured by high resolution respirometry (Oroboros oxygraphy) in the permeabilized muscle fibers.

We show that the lowest nitrite concentrations 0.1 and 1 μmol/L, extend the fly lifespan by 8.75% and 15%, respectively. We found no statistically significant difference in mitochondrial respiration following nitrite treatment. Although nitrite did not affect body weight after 30 days of feeding, we found that nitrite-fed flies had a higher thorax protein content compared to their control counterparts, possibly indicating an increased muscle mass.

In conclusion, we demonstrate a pro-longevity effect of dietary nitrite in female flies at low concentrations. The mechanisms underlying this effect remain to be determined but do not seem to be related to effects on mitochondrial respiration.

P008-T | Mitochondrial dysfunction is linked to an altered mitochondria phospholipidomic profile in non-alcoholic fatty liver disease

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Mitochondrial and oxidative stress-related alterations are crucial factors in the development and progression of Non-Alcoholic Fatty Liver Disease (NAFLD). Although the mechanisms underlying the disease progression are still under debate, some authors have suggested the role of mitochondrial membrane composition influencing mitochondrial dysfunction and hepatocyte damage. Thus, using a diet mimicking the dietary habits of the Western society, we tested the effects of a standard chow diet (C), high-fat (HF), high-sugar (HS) and a combined high-fat and high-sugar (HFHS) diet to address mitochondrial-related alterations in NAFLD. C57BL/6J mice were fed with the above described diets for 16 weeks. Isolated liver mitochondria were used to assess the effect of steatosis on mitochondrial bioenergetic parameters. Mitochondrial lipid classes were assessed through Thin-Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS).

In comparison with the standard chow diet, HF-, HS- and HFHS-diets were shown to decrease oxygen consumption and decrease the capability to generate a mitochondrial membrane potential. Those alterations were accompanied by a significant increase of H_2O_2 production by liver mitochondria of mice fed with the HFHS diet. In parallel with those alterations, mitochondrial cardiolipin content is increased whereas PC/ PE ratio is decreased in HS- and HFHS-fed groups. Moreover, we observed a significant enrichment of monounsaturated fatty acids (MUFA), which are known to be prone to oxidation. In fact, this is in agreement with increased lipid peroxidation levels observed in NAFLD mice.

This work showed that Western society diets cause an altered mitochondrial function which can be attributed to an altered mitochondrial phospholipids composition and a higher susceptibility to ROS-oxidative effects.

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P009-T | Human platelets show a age-dependent decrease in mitochondrial function, but only in a subpopulation of blood donors

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Background: Human platelet concentrates are widely used for life-saving transfusions in patients with thrombocytopenia. Prior to transfusion, platelets can be stored in the blood bank at room temperature for up to 7 days, but during storage acidification occurs, the extent of which correlates with a decline in platelet function. The aim of our study was to investigate whether donor characteristics have an influence on this platelet storage lesion.

Materials and methods: Human platelets were isolated from whole blood donations from single donors by the buffy-coat method and stored for 8 days. In the first (prospective) study, donors were selected on the basis of previous pH data included in the blood bank registries. In follow-up studies, random donors differing in age were selected. pH, lactate and glucose were determined using a blood gas analyzer and the mitochondrial membrane potential (MMP) was assessed by loading the platelets with the fluorescent dye JC-1.

Results: The prospective study indicated that historical data on pH changes during storage were predictive of acidification, showing a clear donor influence on this parameter. Lactate production was 40% higher in the group previously designated as "rapid acidifiers", and the MMP at day 8 was significantly lower. Glucose consumption was 55% higher, indicating increased glycolytic activity to compensate for decreased mitochondrial activity. In the second study it was observed that in donors younger than 30 years, only 1 out of 15 donors could be classified as rapid acidifier, in contrast to 10 out of 22 donors older than 45 years.

Conclusions: Our results show that in human platelets changes in mitochondrial activity are compensated by an (unwanted) increase in glycolytic activity. These changes come with increasing age, but occur only in a subpopulation of individuals. The factors determining the reprogramming of metabolism in individuals remain to be determined.

P010-T | Mitochondrial DNA damage associates with long-term outcome after sepsis

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Sepsis is the leading cause of death among patients admitted to the intensive care unit, with a mortality rate of 20%-30%. In addition, survivors of sepsis are at high risk for the early development of age-related diseases and mortality. Although virtually nothing is known about the pathogenesis of late morbidity and mortality after sepsis, the key might be the induction of mitochondrial DNA (mtDNA) damage due to the mitochondrial dysfunction associated with sepsis. Here, we determined whether sepsis leads to mtDNA damage by analyzing oxidation of DNA, levels of circulating mtDNA and mtDNA damage from patients with infection/sepsis admitted to the hospital. Sepsis is associated with oxidation of DNA and RNA (urine), as well as increased levels of circulating mtDNA. Damage of mtDNA, measured as the ND4-deletion ratio (1/[ND4]:[CytB]) is increased in sepsis. MtDNA damage is strongly and independently associated with long-term mortality. Taken together, mitochondrial dysfunction in sepsis not only seems to influence acute outcome, but may also underlie the increased morbidity and mortality among survivors of sepsis. Therefore, strategies to preserve mitochondrial homeostasis may be key to improve both short- and long-term outcome in sepsis.

P011-T | Mitochondrial damage induced by oxygen-glucose deprivation in the rat barrel cortex in vitro

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Mitochondrial damage is one of the key mechanisms of neuronal death during cerebral ischemia. We explored ultrastructural and functional mitochondrial changes induced by oxygen-glucose deprivation (OGD) in slices of the rat barrel cortex in vitro. Electron microscopy was performed in brain slices which experienced thirty minutes of OGD. Ultrastructural neuronal damage was overall similar to that observed in the ischemic brain tissue in vivo, although the level of edema, which manifested by formation of large clear intracellular spaces was more prominent in the post-OGD slices in vitro. Compared to control slices, which displayed

normal appearance of mitochondria, post-OGD slices showed prominent alterations in mitochondrial structure. Neuronal soma mitochondria displayed increase in size, electron transparent matrix and expanded cristae. Mitochondria of swollen dendrites in the post-OGD tissue were also larger and had an irregular or toroidal shape indicating a disconnection of the electron transport chain; they also displayed expanded cristae. However, part of dendrites were not swollen in the post-OGD tissue, and their mitochondria displayed less prominent changes: they were larger in size and displayed condensed cristae but maintained round shape, which could indicate their adaptation to survival. Along with these findings, post-OGD slices also displayed poor staining with 2,3,5-triphenyltetrazolium chloride, a marker of mitochondrial function. Thus, OGD induces ultrastructural and functional mitochondrial impairments in brain slices in vitro. This model could be useful for exploration of the mechanisms of ischemia-induced of mitochondrial damage and their roles in neuronal death.

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P012-T | Metabolic characterization of a novel pluripotent-paused state

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Background: Embryonic diapause is a conserved reproductive strategy in which development arrests at the blastocyst phase. Recently mTOR inhibition was proven to induce diapause on mouse blastocysts and a paused-like state on mouse embryonic stem cells (mESCs) culture. Due to the potential applications of this technology, and because the metabolic state of these paused-mESCs was never addressed, we aimed to further characterize this new paused-pluripotent state, focusing on its glycolytic and oxidative metabolic function.

Materials and methods: mESCs in two different pluripotent-states were exposed to the mTOR inhibitor INK-128 (mTi) and culture proliferation, pluripotency status and energy-related metabolism were evaluated.

Results: INK-128 significantly decreased mESC culture growth through cell cycle modulation, without inducing apoptosis or altering the pluripotency status of the cells. A decrease in glycolytic and mitochondrial oxidative rates was observed, coupled with a decrease in glucose and pyruvate uptake. Unexpectedly, overall protein expression was not affected.

Conclusions: mTi can induce a diapause-like state in mESC in vitro culture, by slowing proliferation, without affecting the pluripotency status of the culture. Interestingly, paused mESCs present a glucose-related hypometabolic profile, which is a hallmark of diapaused blastocysts; with decreased glycolytic and oxidative metabolism and decreased nutrient uptake. However, INK-128 was not enough to inhibit mTOR translational function; which suggests that this paused state is primarily induced by cell cycle and metabolic modulation rather than translational suppression.

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P014-T | Internalisation of lipopolysaccharide initiates mitochondrial dysfunction

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Sepsis is the leading cause of death among patients admitted to the ICU. Recent studies identified mitochondrial dysfunction as a key player in the development of multiple-organ dysfunction syndrome in sepsis. Mitochondrial dysfunction is characterized by a reduction of the mitochondrial membrane potential (MMP), followed by increased oxidative stress and loss of ATP generation. Yet, the mechanisms that lead to a loss of MMP are still undisclosed. To reveal the underlying molecular mechanisms that lead to mitochondrial dysfunction in sepsis, we challenged human umbilical vein endothelial cells (HUVEC) and isolated mitochondria with lipopolysaccharide (LPS). Incubation of HUVEC with LPS results in loss of MMP, while increasing electron transport chain (ETC) complex activity and mitochondrial reactive oxygen species production, combined with reduced levels of oxygen consumption and ATP production. The effects of LPS are likely mediated by direct interaction of LPS with

mitochondria, as flow cytometry reveals LPS accumulation in mitochondria, blockade of NF-κB signalling does not preclude mitotoxic effects of LPS and similar effects were found in isolated mitochondria. In conclusion, LPS seems to induce mitochondrial dysfunction by a direct interaction with the organelles. As a consequence, precluding LPS internalization may provide a novel target to maintain mitochondrial function in sepsis, and potentially optimize outcome.

P015-T | Unraveling the mechanism throughout mitochondriotropic cinnamic acid antioxidants increase cellular stress responses in HepG2 cells

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Background: Mitochondrial reactive oxygen species (ROS) can act as physiological mediators of stress responses or instead as damaging oxidizing molecules when produced in excess. We previously developed mitochondrial-directed antioxidants based on naturally-occurring phenolic acids, such as hydroxycinnamic acids (HCA) (AntiOxCINs). We hypothesize that AntiOxCINs can modulate the cellular metabolic activity throughout the improvement of mitochondrial function.

Material and methods: Herein, we studied the effects of the novel mitochondriotropic agents (AntiOxCIN4 and AntiOxCIN6) on human hepatoma-derived HepG2 cell line by measuring their effects on oxidative stress, mtDNA copy number, oxygen consumption, glutathione (GSH) levels, lactate production and mitophagy after 48 hours of treatment. Data presented are means ± SEM of four independent cell experiments. Significance was accepted with $P < 0.05$.

Results: Upon a short-term deregulation of mitochondrial function, AntiOxCIN4 increased 1.6-fold basal respiration and 1.2-fold the extracellular acidification rates, followed by a ROS-dependent stimulation of endogenous antioxidant defense system and mitochondrial biogenesis, as measured by a significant increase of 20% in GSH concentration and mtDNA copy number. AntiOxCIN4 treatment promoted an efficient removal of potential damaged mitochondria by triggering cellular quality control mechanisms, such as mito and autophagy. AntiOxCIN6 did not affect mitochondrial function although promoted a 1.5-fold increase in lactate production, suggesting the triggering of a more active glycolytic flux.

Conclusions: Mitochondriotropic antioxidants based on dietary scaffolds can modulate cellular metabolic activity throughout the improvement of mitochondrial function, which increase their usefulness as therapeutic agents in the treatment of oxidative stress-related conditions.

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P016-T | Mitochondriotropic antioxidants based on dietary phenolic acids: Potential effect against chemical-induced skin senescence

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Background: Age-related skin structural changes involves disruption of mitochondrial function, including increased oxidative stress. Polyphenols such as hydroxycinnamic (HCA) acids present antioxidant properties which are limited in the context of skin aging. The major obstacle for their beneficial effects is their limited skin penetration and accumulation in mitochondria. To overcome the latter limitation, novel mitochondriotropic antioxidants have been synthesized and validated in several cell models and isolated mitochondrial fractions.

Material and methods: Normal human dermal fibroblasts (NHDF) were used to test the toxicological profile of novel mitochondriotropic cinnamic derivative antioxidants, as well as their short- (1 hour), mid-(24 hours) and long-term (48 hours) antioxidant effects in an in vitro model of oxidative stress (H_2O_2) by measuring cell viability. Toxicity and antioxidant profiles were compared with parental cinnamic acid as well as with resveratrol, and the mitochondria-directed antioxidant MitoQ10. We performed 4 independent experiments and considered differences with $P < 0.05$ as statistical significant.

Results: The novel mitochondriotropic antioxidants showed dose-dependent cytotoxic effects that were only relevant for concentrations above those where antioxidant activity was observed (50 μmol/L). MitoQ10 was the most toxic antioxidant studied, with toxicity apparent for 0.39 μmol/L. From the compounds tested, AntiOxCIN4 prevented by 32.6% and 24.6% H_2O_2-induced fibroblast loss of viability in short- and long-term incubations, respectively. During med-term incubation, the protective effect was similar to resveratrol and

MitoQ10, but it was ~40% significantly better comparatively to the parental galloyl cinnamic acid (15.4%).

Conclusions: Given the increased need for solutions to target skin aging, the novel molecules, especially AntiOxCIN4, may be used as anti-skin aging active ingredients in topical skin products.

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P017-T | Metabolic fluxes of human urine-derived cells are comparable in young and old donors

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Background: Competent human cells which can be used as surrogate models for disease, in vitro interventions, or regenerative medicine have been intensively researched. Human urine contains a small percentage of cells with proliferative capacity. These urine-derived cells (UDCs) are non-invasively collected and present some features of mesenchymal stem cell phenotypes. Herein, we tested the hypothesis that age impacts on UDCs mitochondrial and metabolic functional parameters.

Material and methods: UDCs were isolated from female donors and divided in two groups (young: 22-35 years old, n = 15 total; old: 70-94 years old, n = 14 total). Mesenchymal and hematopoietic stem cell markers and osteogenic differentiation were studied by flow cytometry and Alizarin Red S staining, respectively. ATP was determined using the CellTiter-Glo Luminescent Cell Viability Assay. Oxygen consumption (OCR), extracellular-acidification (ECAR) rates, and Bioenergetic Health-Index (BHI) were measured with the Seahorse XFe96 Extracellular Flux Analyzer. Polarized mitochondria morphology was imaged by MitoTracker-Red-CMXRos and confocal microscopy. Data obtained was compared by using Mann Whitney test.

Results: UDCs from young and older donors were positive (≥96%) for CD44, CD73, and CD24. The percentage of positive cells for CD90 and CD105 was variable and ≤1% for hematopoietic stem cells markers. Alizarin Red S staining after 28 days of incubation with osteogenic medium, was positive. ATP levels showed no differences between the two groups. Regarding metabolic fluxes, OCR, ECAR and BHI results were similar in the two age groups, except for basal ECAR which was decreased in the older group, suggesting a lower glycolytic rate. A larger cell population with fragmented polarized mitochondria was observed in the old group.

Conclusions: UDCs present mesenchymal stem cell markers and are a non-invasive cell surrogate for investigating interventions or metabolic profiling. Aging is not associated with a change in most bioenergetic parameters measured.

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P018-T | Mitochondrial dysfunctions of adipose tissues for colorectal cancer patients from the redox state measurements

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Background: Obesity is the second highest risk factor for cancer after tobacco smoking but the mechanisms underlying this linking are practically unknown. One of the targets sensitive to damaging influence of colorectal cancer (CRC) tumor in obesity is mitochondria of adipocytes.

Material and methods: Redox state -superoxide (SO) generation rate, activity of complex I in electron transport chain (ETC) of mitochondria and of dinitrosyl iron complexes by electron paramagnetic resonance; activity of matrix metalloproteinase (gelatinase) MMP-2 and MMP-9 by gel zymography of adipose tissues (AT) from 46 patients, 64.3 y.o. with CRC (II-III stages, pT2–3N0–2M0) in the AT adjacent to tumor (ATAT) and at a distance of 3 cm from the tumor (ATD) was investigated. We have incubated the AT species with the tumor necrosis factor alpha (TNF-alpha) to follow its influence on the measured values. As a control, normal AT (NAT) obtained during the liposuction was used.

Results and conclusion: Tumor-induced changes in mitochondrial ETC of ATAT, particularly for Complex I, lead to the enhanced SO generation and consequent oxidative modifications of DNA in ATAT (up to 6.1 times higher than that in NAT and 3.7 times higher than that in ATD, $P < 0.05$).

Gelatinase activity in ATAT is significantly higher than in ATD. A considerable effect of TNF-alpha on ATAT and ATD (but not on NAT, i.e., only on the tissues where the reprogramming of metabolism has already occurred under the influence of tumor) manifested in increase of cellular hypoxia, gelatinase activity, and SO generation rate is observed. The results can be used for better understanding the mechanism(s) of metabolic symbiosis of tumor and AT as well as serving as a basis for new therapeutic approaches.

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P019-T | Expression of nuclear and mitochondrial mismatch repair genes (MMR) in colorectal carcinoma: A study of North Indian population

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Background: Colorectal cancer (CRC) is second and third most commonly diagnosed cancer among women and men worldwide respectively. Deficiencies in DNA mismatch repair (MMR), which corrects base mismatches and small loops, are associated with DNA microsatellite instability, mutations, and cancer.

Objective: To study the expression of Nuclear MMR Protein (MLH1, MSH2, PMS2 and MSH6) and Mitochondrial repair protein YB1, TFAM and its Clinicopathological correlation in CRC patients in north India.

Methods: A prospective study was conducted on histologically proven patients of adenocarcinoma of colorectum in a tertiary care hospital in north India between May 2014 and May 2018. MMR Protein loss was determined by using Immunohistochemistry for the above proteins.

Results: 77 patients (49 males and 28 females) underwent resection for CRC with the median age of 52 year (16-81 years). 44% of the patients (n = 34) were younger than 50 years of the age. Five patients had associated history of malignancy in the family. 42 (55%) patients had right colon cancer, 15 (19%) left colon cancer and 20 (26%) rectal cancer. Histology revealed well differentiated tumour in 26, moderately in 15 and poorly in 36 patients. MMR protein loss was seen in 23 (30%) patients. Seven (46%) of these patients were less than 50 years of age. Combined loss of MSH2 and MSH6 was seen most commonly and it was found in 6 patients. 18 (78%) patients with MMR protein loss had tumour located proximal to the splenic flexure compared to 5 (22%) located distal to the splenic flexure. The immunohistochemical analysis of Mitochondrial Protein YB1 and TFAM is to be done.

Conclusion: This study revealed that there was less than 30% MMR protein loss in colorectal cancer patients in north Indian population. The loss was most commonly seen in right sided colon cancer than left.

P020-T | Hepatic oxidative response to maternal obesity during pregnancy in an ewe model

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Background: Obesity prevalence is rising worldwide, accompanied by increased incidence of nonalcoholic fatty liver disease (NAFLD). NAFLD is characterized by hepatic fat accumulation, metabolic impairment and mitochondrial dysfunction. Pregnancy represents a challenge to female organism and metabolism. For a compromised liver, pregnancy-associated increase in energy and nutrients requirements might jeopardize mother's hepatic metabolism. The goal of this work is to characterize the effects of maternal obesity (MO) during gestation in the hepatic tissue. Due to the liver anatomy, the metabolic response to MO will be characterize in a lobe-dependent manner.

Methods: We used a MO ewe model (Rambouillet-Columbia) induced by an excess of diet (150% of recommended nutrition) which started 60 days before the conception. At 90% gestation time, animals were sacrificed, and the maternal hepatic tissue collected. Hepatic redox state and antioxidant defenses were analyzed. Data were compared between MO and control groups (n = 8 and 10, respectively), using the most pertinent statistical test with $P < 0.05$ considered statistically significant.

Results: MO increased lipid peroxidation, with greater impact in the right lobe (RL) when compared with the left lobe (LL) (81% vs 36%). The enzymatic antioxidant defenses were modulated in a lobe-dependent manner: MO showed 30% reduced catalase activity and increased GSH levels in 30% in the LL, while in the RL, MO led to a decrease of 50% in GSH levels and an 80% increase in SOD activity.

Conclusions: MO during pregnancy stage is characterized by specific alterations in liver redox state to accommodate the pregnancy-challenge with possible implications in the

hepatic mitochondrial metabolism. Our results pointed lobe-specific adaptations during this condition.
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P021-T | Redox signaling and metabolism modulation of human fibroblasts by mitochondria-targeted polyphenolic constructs

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Background: Homeostatic mitochondrial function and redox balance is essential for proper cellular survival or death signaling. Thus, mitochondria pose as strong targets for therapeutic intervention strategies. Antioxidants based on naturally-occurring phenolic acids have shown great pharmacological interest due to their intrinsic redox regulating properties. Despite their limited use as therapeutic agents due to pharmacokinetic drawbacks, they are useful scaffolds in drug discovery processes. Novel mitochondriotropic agents developed by our research group (AntiOxCINs and AntiOxBENs) based on hydroxycinnamic and hydroxybenzoic acids, respectively exploit the covalent bonding of a lipophilic cation to drive specific accumulation in mitochondria.
Materials and methods: Herein we studied the time-dependent effects of mitochondria-targeted polyphenolic constructs (AntiOxCIN4 and AntiOxBEN2) on cell metabolism by measuring intra- and extracellular levels of lactate, glucose, alanine and acetate in normal human dermal fibroblasts (NHDF) through nuclear magnetic resonance (NMR) techniques. Data are the mean ± SEM of six independent experiments. Statistical significance was attained comparing the results with the control group using a one-way ANOVA followed by the Dunnett post-test.
Results: AntiOxCIN4 ($*P < 0.05$) and AntiOxBEN2 ($****P < 0.0001$) significantly increased the production of lactate when compared to control. However, the corresponding increased glucose consumption was only observed in AntiOxCIN4-treated cells. After 72 hours treatment, intracellular lactate and acetate levels were significantly increased in both AntiOxCIN4 and AntiOxBEN2 ($*P < 0.05$), while no differences were observed between alanine levels, when compared to control. Thus, the intracellular lactate/alanine ratio was increased in cells treated with AntiOxBEN2 ($*P < 0.05$), suggesting an increased demand for NAD+ under this treatment.
Conclusions: Our preliminary work shows that the novel mitochondriotropic antioxidants are able to induce a metabolic adaptation in NHDF cells by increasing their glycolytic flux.
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P022-T | Metabolic remodeling of dermal fibroblasts by a novel mitochondria-targeted hydroxybenzoic acid derivative

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Background: Polyphenols such as hydroxybenzoic acids (HBA) have been extensively studied due to their antioxidant effects, presenting different mechanisms at several subcellular compartments. Mitochondria are central to cellular metabolism, making these organelles strong targets for therapeutic intervention strategies. Attempts to modulate mitochondrial function in a diversity of diseases spurred active drug discovery efforts for developing new mitochondriotropic antioxidant agents. Based on this, we developed a novel mitochondriotropic agent (AntiOxBEN2) based on HBA, exploiting the covalent bonding of a lipophilic cation to drive specific accumulation in the mitochondria.
Material and methods: We studied the effects of AntiOxBEN2 (12.5 μmol/L) on human normal dermal fibroblasts (HNDF), namely by measuring cell metabolic activity, mitochondrial oxygen consumption and DNA, protein amount of mitochondrial respiratory chain subunits, ATP levels, extracellular pH and cell proliferation. AntiOxBEN2 antioxidant effect was also evaluated. Statistical significance of treated vs control group was tested by one-way ANOVA.
Results: Although AntiOxBEN2 had no effect on cell metabolic activity, a significant decrease in mitochondrial oxygen consumption ($****P < 0.0001$), paralleled by a decrease in mtDNA copy number was observed. No changes were observed neither on oxidative phosphorylation protein subunits expression nor on ATP levels. Interestingly, AntiOxBEN2-treated

cells significantly increased extracellular acidification rate (****$P < 0.0001$), as well as cell proliferation (*$P < 0.05$), increasing cell resistance to oxidative stress (*$P < 0.05$).

Conclusions: AntiOxBEN2 may trigger an adaptative response of cells, a process that can protect them against subsequent stress-inducing events.

Mitochondria-targeted antioxidant based on hydroxybenzoic acid can stimulate cell compensatory responses and contribute to tissue protection, inhibiting directly or indirectly an excessive mitochondrial reactive oxygen species production.

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P023-T | Non-alcoholic fatty liver disease (NAFLD) in vitro: Characterization and comparison of several in vitro models using human hepatic cells

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Background: Non-alcoholic steatohepatitis (NASH), the progressive form of non-alcoholic fatty liver disease (NAFLD), is a worldwide health problem with no effective treatment, offering a considerable market opportunity for developing novel drugs. Several models mimetize in vitro NAFLD but significantly differ in nature, amount and fatty acid (FA) overload incubation, making data comparison difficult. Herein, we characterize and compare different FA overload strategies, with particular focus in measuring mitochondrial function, to attain a feasible model for high throughput screening of possible therapeutic agents.

Material and methods: We studied the time-dependent effects of FA overload, namely palmitic acid (PA; 500 μmol/L) and a mix of free FA (FFA; 250 μmol/L) in the presence and absence of fructose (F; 10 mmol/L) on cell viability, lipid accumulation, mitochondrial oxygen consumption, mitochondrial DNA copy number, intracellular reactive oxygen species (ROS) and ATP levels of human hepatocarcinoma cells (HepG2). Statistical significance of treated vs control group was made by using a one-way ANOVA.

Results: Both PA and FFA treatment induced a time-dependent significant increase in intracellular lipid accumulation (~240% and ~230%, respectively) and ROS levels (~140% and ~180%, respectively) paralleled by a ~55% and ~70%, respectively significant decrease in mitochondria oxygen consumption. FFA treatment significantly increased mtDNA copy number (~110%) without affecting intracellular ATP levels, while PA treatment significantly decreased intracellular ATP levels (~80%) without affecting mtDNA copy number. In general, the presence of fructose in both PA- and FFA-treated cells did not affect any of the several steatohepatitis-associated hallmarks.

Conclusions: FFA seems to be the best model to study NAFLD/NASH mechanisms in vitro, as it induces several steatohepatitis-associated hallmarks without affecting cell viability.

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P024-T | Effects of physical exercise, during pregnancy, on cardiac mitochondrial function in a rodent model of high-fat high-sugar diet-induced gestational diabetes

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Introduction: Gestational Diabetes (GD) affects 5%-10% of pregnancies, characterized by increased insulin resistance and mild hyperglycaemia, rising the risk of developing type 2 diabetes (T2D). Cardiac diseases are frequent T2D complications, involving mitochondrial-related dysfunction, which is counteracted by physical-exercise (E). Whether E during pregnancy influences cardiac mitochondrial during GD is not completely understood.

Methods: Female Sprague-Dawley rats were fed with control-diet (C) or high-fat-high-sugar-diet (HFHS) and distributed as follows: non-pregnant or pregnant (NP/P) control (C) or HFHS diet, sedentary (S) or E. Mothers (19-weeks-old) were sacrificed seven-weeks after weaning. Body, heart weights and blood glucose were evaluated.

Cardiac mitochondria oxygen consumption and membrane potential were measured with a Clark-type and TPP+-electrode, respectively.

Results: HFHS or E caused no significant alterations in maternal weight, even during pregnancy. Also, no significant differences between groups were found in heart mass. Oral glucose tolerance tests showed no significant differences between HFHS and C groups before pregnancy. However, after becoming pregnant, HFHS (n = 7) increased ~20% glucose levels, when compared with C (n = 9). Regarding mitochondrial bioenergetics, a ~35% increase in state 2 respiration was observed in pregnant-HFHS-exercised (P-HFHS-E, n = 6) when compared with pregnant-HFHS-S (P-HFHS-S, n = 6). P-HFHS-E exhibited decreased uncoupled respiration and increased ADP phosphorylation lag phase, when compared with P-C-S animals (n = 3). Also, a ~40% lower respiratory control ratio in non-pregnant-HFHS-exercised animals (NP-HFHS-E, n = 3) was observed, which resulted from higher basal state (~30%) and reduced ADP-induced phosphorylation with increased phosphorylative lag phase (~20%), and ~40% increase in ADP-induced depolarization, vs NP-C-S (n = 5). Moreover, NP-HFHS-E group showed ~30% lower uncoupled respiration vs NP-C-S.

Conclusions: HFHS and E, isolated or combined, resulted in altered cardiac mitochondrial function, exacerbated by pregnancy.

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P025-T | Human skin fibroblasts from sporadic Parkinson's disease patients as a surrogate to measure the efficacy of novel mitochondria-directed antioxidant therapy

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Introduction: Parkinson's Disease (PD) is an incurable neurodegenerative disorder characterized by the death of dopaminergic neurons in substantia nigra pars compacta. Several studies suggest that mitochondrial dysfunction and oxidative stress are hallmarks of dopaminergic neurodegeneration of PD. In this context, mitochondria-targeted antioxidant therapies may have great promise in the prevention and/or treatment of PD. The objective of our work was to (a) use human skin fibroblasts (HSF) from sporadic PD (sPD) patients as a surrogate model to detect early metabolic and mitochondrial alterations observed in PD, and (b) to evaluate the effects of novel mitochondria-targeted antioxidants to improve the PD phenotype.

Materials and methods: HSF from sPD patients and respective matched controls were characterized by measuring proliferation rates, metabolic viability, ATP levels, mitochondrial polarization, mitochondrial bioenergetics, oxidative stress, and SOD2 activity. Using the same methods, the effects of AntiOxCIN, a novel phenolic acid-based mitochondria-targeted antioxidant were evaluated.

Results: Our results showed that HSF from sPD have a 45.2 ± 5.0% decrease in metabolic viability, 16.7 ± 4.8% decreased oxygen consumption rate, 14.8 ± 3.9% lower mitochondrial polarization and 40.6 ± 3.7% lower ATP levels, when compared to the respective controls. Also, 40.7 ± 11.8% increased mitochondrial oxidative stress and SOD2 activity were measured in sPD fibroblasts. HSF from sPD treated with non-toxic concentrations of AntiOxCIN showed 34.4 ± 9.6% improved mitochondrial function, converting HSF from sPD physiologically more similar to the matched controls.

Conclusions: We demonstrated that impaired mitochondrial bioenergetics and the defective metabolism that typically characterizes the neurodegenerative process in PD can be detected in HSF from sPD patients. Moreover, treatment with AntiOxCIN increases metabolic viability of HSF. HSF may represent a minimally invasive tool to study altered metabolism and new treatment strategies in PD.

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P027-T | Influence of SOD1 mutation on lymphoblast respiration and glycolytic rates from amyotrophic lateral sclerosis patients

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Background: Mitochondrial dysfunction is a critical factor for neurodegeneration in amyotrophic lateral sclerosis (ALS), particularly in familial mutant SOD1 (mutSOD1). We studied respiratory and glycolytic fluxes in lymphoblasts from ALS patients with determined and undetermined SOD1 mutations to confirm possible alterations in those metabolic pathways.

Methods: Lymphoblasts from 2 patients with mutSOD1 and from 2 patients in which that mutation is undetermined (undSOD1), as well as 2 sex and age-matched controls were obtained from Coriell Cell Repository. Oxygen consumption (OCR) and extracellular-acidification (ECAR) rates were measured using the Seahorse XFe96 Extracellular Flux-Analyzer. ATP levels were assessed by bioluminescence assay. Data are expressed as mean ± SEM and comparisons between groups performed by One-Way ANOVA and Tukey post-test, P-value < 0.05 as significant.

Results: Lymphoblasts from patients with undSOD1 presented a more energetic phenotype than lymphoblasts from mutSOD1 patients or control lymphoblasts matched to sex and age. Spare respiratory capacity presented higher values in lymphoblasts from patients with undSOD1 than in control lymphoblasts ($P < 0.01$) or in lymphoblasts from patients with mutSOD1 ($P < 0.01$), indicating a higher mitochondrial capacity of these cells to respond to energetic demands. Lymphoblasts from patients with undSOD1 presented also higher values of glycolytic ATP production rate in women ($P < 0.05$) and an observed trend in men, suggesting possible activation of glycolysis. Lymphoblasts from patients with mutSOD1 showed lower ATP production-linked OCR and mitochondrial ATP production rate when compared to undSOD1 lymphoblast ($P < 0.05$), possibly because of mitochondrial impairment.

Conclusions: Our data show a metabolic activation in lymphoblasts of ALS patients with undSOD1, while lymphoblasts from ALS patients with mutSOD1 have an impairment of mitochondrial function. Lymphoblasts from ALS patients present mitochondrial alterations and can be used to study mutSOD1 effects on mitochondrial function.

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P028-T | Impact of second-generation antipsychotics on mitochondrial bioenergetics after acute treatment in rats

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Background: Schizophrenia is a highly debilitating mental disease and its early diagnosis is of crucial importance since a prompt treatment can influence its clinical development. Therefore a long-term therapy is essential for the life quality of schizophrenic patients. Second Generation antipsychotics (SGAs) have proven to be effective in the treatment of several psychotic disorders, including schizophrenia. However, the commonly used antipsychotic drugs can cause a list of significant side effects, such as weight gain and type 2 diabetes, that induces many patients to quit the treatment. The underlying molecular pathways for these side effects are not well described and their onset might be triggered by a complex interplay between both central and nervous systems. Being so sensitive to any kind of metabolic alteration, mitochondria are an interesting target when it comes to Antipsychotic Induced Weight Gain (AIWG) studies.

The purpose of this study was to evaluate the in vivo acute effects of olanzapine (OLZ) and aripiprazole (ARI) after one single dose administration on the metabolic profile of male rats, as long as their impact on the mitochondrial bioenergetics of different organs.

Materials and methods: 42 male wistar rats have been randomly divided into three groups: Control, OLZ and ARI, (n = 14) and administered a single dose of the respective drug one hour prior the sacrifice. Respirometry experiments were performed on a O2k machine (Oxygraph-2k Oroboros Instruments, Innsbruck, Austria) using fresh tissues (white adipose tissue, Liver and Muscle fibers) on the day of each sacrifice.

Results and conclusions: The collected data showed that both OLZ and ARI significantly altered mitochondria respiration in adipose tissue. On the other hand, only OLZ showed to negatively impact mitochondrial function in permeabilised muscle fibers.

Fundings sources: European Union's Horizon 2020 research and innovation programme, Marie Skłodowska-Curie grant agreement 721236-TREATMENT

P029-T | Dual administration of liraglutide and ghrelin recovers brain mitochondrial energy metabolism in the R6/2 mouse for Huntington's disease

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Background: Huntington's disease (HD) is a hereditary, incurable, autosomal dominant neurodegenerative disease whose neuropathological and clinical features may include diabetes and cachexia. We hypothesized that co-treatment with liraglutide (an effective anti-type 2 diabetes (T2D) and anorectic drug) and the orexigenic hormone ghrelin mitigates brain mitochondrial dysmetabolism and cognitive deficits in HD.

Materials and methods: We aimed to analyze the effect of a 2-week subcutaneous co-injection of liraglutide and ghrelin on brain cortical mitochondrial (energy) metabolism and motor function in the early symptomatic R6/2 mouse for HD. These involved the measurement of mitochondrial respiratory chain complexes' activities by colorimetry, and the behavioral paw clasping and open-field tests.

Results: Liraglutide alone or plus ghrelin minimized peripheral T2D characteristics in R6/2 mice. This was accompanied by a liraglutide plus ghrelin-induced stimulation of the R6/2 mouse brain cortical mitochondrial respiratory chain complex IV, and a decrement in lactate and AMP levels that may account for an increase in their brain energy status compared to saline-treated R6/2 mice. However, neither the paw clasping score or the locomotor/exploratory activities were significantly rescued by the treatment in these mice.

Conclusions: Liraglutide plus ghrelin may recover brain cortical mitochondrial energy metabolism, ultimately slowing HD progression. However, this issue deserves further clarification.

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P030-T | Neonatal Rotenone administration induces mitochondria metabolism and biogenesis deregulation leading to synaptic dynamics and behavioral alterations mimicking neurodevelopmental disorder

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The neurodevelopment is an ATP-dependent process and, therefore, relies on mitochondrial function. Moreover, it is known that disruption in any pathway related to neurodevelopment can lead to neurodevelopment disorders. Thus, the aim of this study was to evaluate the involvement of mitochondrial dysfunction in molecular and behavioral alterations related to neurodevelopmental disorders. For reach this goal, we treated wistar puppies rats (P) from P5 to P11 with Rotenone (Rot., 0.1 e 0.5 mg/kg), an inhibitor of the complex I of mitochondria respiratory chain, Saline and DMSO (Vehicle). At 60 days of age (P60) we evaluated the levels and expression of CBP, CREB, Pgc1 alpha, Nfr1, Nfe2l2, Synaptophysin, PSD-95 and Rest. In addition, to verify possible behavioral alterations, we measured total motor activity (open field) and social interaction. Results show, when compared to control group (100% ± SEM), a decrease in the levels of CBP (for groups Rot 0.1 mg/kg: 63.35% ± 5.24 $P < 0.05$ and Rot 0.5 mg/kg: 42.74% ± 6.45 $P < 0.01$), and CREB (for Rot 0.5 mg/kg: 57.61% ± 9.42 $P < 0.05$). However, the levels of Synaptophysin was decreased for group Rot 0.5 mg/kg (12.84% ± 3.68 $P < 0.01$), and PSD-95 was increased for groups Rot 0.1 mg/kg (188.7% ± 7.16 $P < 0.01$) and Rot 0.5 mg/kg (211.9% ± 15.78 $P < 0.01$). Moreover, there is a significant reduction in the expression of Pgc1alpha, Nrf1and Nfe2l2 after Rot 0.1 mg/kg treatment, in addition to an increase in Rest for Rot 0.5 mg/kg. Regarding motor activity, Rotenone treatment induced an increase in this parameter (0.1 mg/kg: 138% ± 7.99 $P > 0.01$; 0.5 mg/kg: 125.6% ± 7.27 $P > 0.05$). Rot 0.1 mg/kg (69.63% ± 7.93 $P > 0.05$) and Rot 0.5 mg/kg (50.21% ± 4.05 $P > 0.001$), on the other hand, induced a decrease in social interaction. We concluded that neonatal Rotenone administration can promote behavior and molecular alterations that persists at early adulthood; this phenotype is

present in animal models of neurodevelopmental disorders.
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P031-T | Distinct role of Rab39a expression in colorectal cancer

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Background: Rab proteins control the endocytic vesicle trafficking. Dysregulation of several Rab proteins have been associated with promoting tumorigenesis. The role of the Rab proteins in regulation of inflammation was demonstrated where the Rab39a was shown stimulating the Nod-like receptors (NLRs). Interestingly, stimulation of the NLRs can suppress the growth of colorectal cancer (CRC). However, the role of Rab39a in CRC suppression remains unknown. Therefore, we sought to determine the association between Rab39a expression and NLRs caused tumor suppression in CRC in vitro.

Material and methods: HCT-116, a colorectal cancer cell line, was treated with NLRs inhibitor (VX765) or activator (Nigericin). The effect of VX765 and Nigericin on NLRs was analyzed by detection of IL-1β, IL-18, CASP1, CASP8 and NLRP3 transcripts using RT-qPCR. NLRP3 protein expression was analyzed using western blot, while IL-1β and IL-18 secretion was detected using ELISA. The effect of VX765 and Nigericin on RAB39A expression was analyzed using RT-qPCR. The effect of VX765 and Nigericin and RAB39A expression on tumor cell vitality were analyzed using Annexin V, cell proliferation assay, LDH assay, JC-1 Assay and a 40Plex cytokine assay. Data was statistically analyzed using Independent sample *T* test and One Way Anova, Post Hoc Tukey test.

Results: As expected, IL-1β, IL-18, CASP1, CASP8 and NLRP3 mRNA levels were suppressed when cells were treated with VX765, while Nigericin upregulated these genes ($P < 0.05$). Rab39a expression was 1.49 ± 0.20 fold decreased in cells treated with VX765. In contrast, expression of RAB39A was 27.18 ± 1.81 fold increased when cells were incubated in presence of Nigericin. Cell viability and proliferation increased in presence of VX765, while it was decreased when cells were treated with Nigericin ($P < 0.05$).

Conclusion: We demonstrated that, high RAB39A expression suppressed CRC though NLRs activation.

Funding sources: This study was supported by RFBR Grant #18-34-01000.

P032-T | The effect of Rab5 on NLRP3 inflammasome activation in colorectal cancer

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Background: Colorectal cancer (CRC) is the third most common cancer diagnosed worldwide. Recently, it has been shown that NLRP3 inflammasome could be a potential therapeutic target to reduce the invasive and metastatic potential of CRC. NRLP3 supports tumor growth by activation and secretion of IL-1β and IL-18 in extracellular vesicles. Rab5, a Rab GTPase, is one of the key regulator of a membrane transport in the early vesicle trafficking. Currently, little is known about the role of Rab5 in NLRP3 inflammasome activation.

Material and methods: HCT-116, a CRC cell line, was transfected with DsRed-Rab5-Wild Type (WT) or DsRed-Rab5-Dominant Negative (DN) plasmids. Protein expressions were confirmed by fluorescent microscopy and flow cytometry. Rab5-WT and Rab5-DN cells were treated with Nigericin to stimulate NLRP3 inflammasome Inflammasome activation was analyzed by detection of IL-β, IL-18, CASP1, CASP8 and NALP3 using qPCR. The effect of inflammasome activation on cell morphology in Rab5-WT and Rab5-DN cells was determined using confocal microscopy. Data was analyzed using One Way Anova, SPSS 20.

Results: Nigericin significantly increased the mRNA levels of inflammasome associated genes (IL-β, IL-18, CASP1, CASP8 and NALP3) as compared to untreated cells in both Rab5-WT and Rab5-DN cells ($P < 0.05$). Nigericin treatment of Rab5-WT cells significantly increased expression of inflammasome related genes as compared to Rab5-DN cells ($P < 0.005$). Particularly, in Rab5-DN cells, IL-18 expression was 9.77 fold higher when compared to untreated cells ($P < 0.003$). Additionally, Nigericin treatment increased (457.76 fold) expression of IL-18 in Rab5-WT cells as compared to untreated cells ($P < 0.039$). Nigericin treatment affected the cell morphology in both Rab5-WT and Rab5-DN cells, where dead cells were detected.

Conclusion: We demonstrated that stimulation of Rab5 activate inflammasome in CRC. We suggest that Rab5 have a potential to be a therapeutic target for CRC immunotherapy.

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P033-T | Moderate exercise training on mammary cancer: A preclinical study

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Background: Mammary cancer is one of the most frequent cancers among women. Lifestyle is commonly appointed as a risk factor for cancer development. This work intended to evaluate the effects of long-term moderate exercise training on mammary cancer chemically-induced in a rat model.

Materials and methods: Procedures followed the European legislation and were approved by the Portuguese Ethics Committee (approval nº008961). Fifty female Sprague-Dawley rats were randomly divided into four experimental groups: MNU sedentary, MNU exercised, control sedentary and control exercised. At 50 days of age, animals from MNU groups received an intraperitoneal injection of the carcinogen of N-methyl-N-nitrosourea (MNU). Animals from exercised groups were trained on a treadmill for 35 weeks (20 m/min, 60 min/d, 5 times/wk). Animals were palpated weekly for the detection of mammary tumor development. At the sacrifice, mammary tumors were collected and processed for histopathological and immunohistochemical analysis. Blood samples were collected to determine the serum levels of inflammatory markers.

Results and conclusions: As expected, animals from control groups did not develop any mammary tumor. Exercised animals developed a lower number of mammary tumors (23 vs 28, $P > 0.05$) and lesions (50 vs 71, $P = 0.056$), when compared with sedentary ones. The malignancy of mammary lesions was higher in sedentary group. The serum levels of C-reactive protein (CRP) and interleukin (IL)-6 were also lower in exercised animals ($P < 0.05$). The immunoexpression of estrogen receptors (ER) α was higher in mammary tumors from exercised animals ($P < 0.05$). Exercise training positively influenced mammary carcinogenesis, by reducing the number and malignancy of lesions, decreasing inflammation, and increasing the immunoexpression of ERα, which suggests a better response for hormone therapy.

Funding: This work was supported by National Funds by FCT—Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019.

P034-T | Resemblance of transcriptome changes in mice diaphragm to the terrestrial neuromuscular disorders after 30-day space flight on Bion-M1 biosatellite

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Background: Weightlessness is one of the deleterious factors acting on humans in space. The effect of weightlessness is mostly manifested in the development of hypogravity motor syndrome (HMS), which is characterized by specific changes in postural and voluntary skeletal muscles, bone tissue. Meanwhile the effect of hypogravity exposure on the breathing musculature is out of scope in the field of HMS.

Material and methods: In the present study mice after 30-day space flight (Space); mice after a 30-day space flight and subsequent 7-day re-adaptation (Recovery); and mice housed in conditions of Earth gravity (Control) were employed. Total RNA from diaphragm samples was used for a full genomic study using Mouse Microarray Kit. The revealed set of differentially expressed genes was analyzed using Gene Ontology and Human Phenotypes databases.

Results: A comparative analysis of the gene expression in mice between "Space" and "Control" groups didn't reveal differentially expressed genes. In comparison of "Recovery" vs "Control" the analysis of differential gene expression revealed 647 up- and 895 down-regulated genes, in comparison of "Recovery" vs "Space" groups — 273 and 580 up- and down-regulated genes, respectively. Functional annotation of differentially expressed genes using the Human Phenotype Ontology database allowed us to obtain terms that resemble terrestrial human diseases. The terms "Weakness of muscles of respiration", "Hyporeflexia", "Skeletal muscle atrophy", "Functional motor deficit", "Abnormal synaptic transmission at neuromuscular junction" reflect disorders associated with condition of skeletal muscle fibers and motor nerve endings of the diaphragm.

Conclusion: The obtained data on the functional activity of the diaphragm genes in mice after orbital space flight could serve as the basis for optimizing physical training protocols and developing of a new approach for preventing of HMS in cosmonauts during long space flights.

Funding sources: The study was funded by grant RFBR 17-04-00385 and supported by Program of Competitive Growth of KFU.

P035-T | Influence of mechanical unloading on the morphofunctional state of the soleus muscle

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During space flights or locomotor pathologies in earth conditions the morphofunctional state of the neuromotor apparatus changes dramatically. One of the main reasons is supposed to be the restriction of support afferentation. In the present work, the morphofunctional state of the m. soleus of rat was evaluated in modeling the restriction of support afferentation of the hind limbs by hanging animals (n = 7) by the tail in the upside down position (≈30°). After 7 days of exposure to the experimental conditions, the isoform composition of titin in the m. soleus and the M-wave parameters were assessed when the sciatic nerve was stimulated. As control data, used data received in the study of intact animals (n = 5).

It was found that in m. soleus of rats after seven days of restriction of support afferentation, there is a decrease in the content of titin N2A isoform relative to the content of myosin heavy chains by 25% ($P < 0.05$). The decrease in the relative amount of titin N2A isoform is accompanied by a 3-fold increase in the relative content of T2 fragment ($P < 0.05$), which indicates a change in proteolysis of the titin N2A isoform of titin. With high-frequency stimulation of the sciatic nerve (50 Hz), a significant depression of the M-response was recorded (the decrement was 42 ± 6%, $P < 0.05$), which indicates a decrease in the reliability of synaptic transmission. Thus, the activity of support receptors determines the morphofunctional properties of the motor system. Restriction of support afferentation initiates morphological changes in the rat's soleus muscle, and also leads to impaired neuromuscular transmission.

This work is supported by the Russian Science Foundation under grant 18-75-10027.

P036-T | The model of artificial neural network, allowing the formation of a motor program generator

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The aim of this study was the search of general rules of constructing of the simple neural networks which was capable to form their own pattern of muscle activity. On the basis of the original software the variants of the neural structures configurations have been tested. These networks can provide the plasticity on the level of conditioning the signals coming from the neural blocks of different functions, which can allow the implementation of the principle of Hebbian plasticity. The principal architecture of dynamic stochastic artificial neural network, provided the facilitation of the execution of the motor program was found.

The present study involved qualitative analysis of neurophysiological data of the principal mechanisms of functioning of relatively simple neural structures. The formal threshold element with formal spike activity without detailed description of the spike shape was chosen as a neuron model. The model of the artificial neural network which provided the simplification of the implementation of the "external" motor program was proposed. The formation of the own pattern generator of muscular activity with the profile of the activity of the prescribed shape for each effectors occurred as a result of repetitions of this motor program. Obtain model allowed us to explain the behaviour of the late responses during frequency stimulation in electrophysiological experiments in situations when performing motor program was familiar to the subject and depended on the context. The described scheme also can explain the necessity of optimal sensory input in restoring pre-existing motor programs, including motor programs after injuries, as well as the necessity of the additional activation or loading for improving of the motor response.

The work supported by RFBR (grant No. 18-44-160032_r_a).

P037-T | Unsupervised learning of activity patterns in the neonatal rat auditory cortex

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Spontaneous activity patterns in the neonatal rat sensory cortex display region-specific features. Here we aimed to compare the spontaneous activity in the auditory (AC) and somatosensory (SC) cortices of P6-7 rats (N = 7) using machine learning approach. We developed a method to differentiate one second long patches of activity in AC and SC using convolutional artificial neural networks. We trained the network on the most salient patches of activity to learn characteristic AC and SC waveforms and then applied it to the whole traces of spontaneous activity in both structures. We found that spontaneous activity in AC specifically displayed long-lasting megabursts of the local field potential oscillations in a wide range from theta to gamma frequencies and multiple unit activity lasting for ≥7 seconds and occurring at a rate of 40 ± 12 megabursts per hour. The AC

activity also expressed discrete ramp-shaped troughs (sharp potentials, SPs) lasting ~100 ms, some of which occurred within megabursts. SPs were preceded by spiking activity in the inferior colliculus at a delay of 20 ± 4 ms. The current-source density analysis of both AC patterns revealed their main sinks in the cortical layer IV. These findings point on the region-specific organization of activity in the developing cortex and suggest that prior to the onset of hearing the AC activity is largely driven by spontaneous activity at the auditory periphery.

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P038-T | Prediction of bioisosteric substitutions using the Condensed Reaction Graph approach

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The prediction of bioisosteric substitution for molecule is extremely important and challenging. The replacement is called bioisosteric if activity of molecule is barely influenced. The substitution of potentially toxic fragments by less dangerous can give insight to safe modifications of drugs and/or hit molecules. It also could be used for focused library generation, scaffold hopping or lead optimization.

The approach for prediction of bioisosteric replacements was proposed. It contains 3 steps: (i) bioisosteric rules extraction, (ii) its application for molecules of interest and (iii) filtering results by model, predicting whether substitution is bioisosteric.

149 890 matched molecular pairs (MMP) with known log IC50 values for 12 biological targets was extracted from ChEMBL. Each MMP was considered pseudo-reaction, transforming one molecule into other. Condensed graph of reaction (CGR) approach was used for MMPs representation. CGR is a representation of reaction as a pseudomolecule that has additional to classical (single, double, etc. bonds and charges) features new types as dynamic bonds and/or charges which show changes within transformation. Rules that correspond to substitution of one chemical functionality by the other were automatically extracted for every biological target. Using ISIDA Fragment or descriptors for CGR was calculated. Random Forest (RF) was used to build classification model for every target. If difference in logIC50 was less than 1 substitution was considered bioisosteric. These models were used as a filter for newly generated CGRs to predict if this substitution is bioisosteric.

The approach was tested on one dataset retrospectively. It was shown that application of the approach to a seed molecule generates set of compounds within which compounds with the same type of activities could be found.

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P039-T | Adverse drug reactions identification in social media posts and electronic health records with neural networks

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Background: Social media posts and electronic health records have become a promising source for detecting adverse drug reactions (ADRs). We introduce a system for automatically detecting of ADRs from natural language texts. The system based on artificial neural networks and consists of two components: named entity recognition (NER) and entity type classification. The NER module aims to extract words and multi-word expressions denoting diseases. Disease type classification is necessary to distinguish drug reactions from drug indications.

Material and methods: The NER module is based on a hybrid Long Short Term Memory (LSTM) and conditional random fields architecture. The classification module is based on Interactive Attention Neural Network (IAN). IAN is the attention-based LSTM network, which takes as an input entity and its context in a sentence. Both entity and context representations interact with each other's attention mechanism to generate the overall representation. Extension of the NER model by the IAN classifier allows to separate the representation of the entity from the context and organize their interaction.

We evaluated the efficiency of the model on CADEC (https://data.csiro.au) and MADE (http://bio-nlp.org/index.php/projects/39-nlp-challenges) corpora. CADEC corpus consists of user-generated reviews about drugs from askapatient.com forum. MADE corpus contain de-identified electronic health record notes of cancer patients.

Results: We evaluated the model using the F-measure, which is the standard quality metric in natural language processing. The model obtained 76.5% and 72.6% F-measure for ADRs class on the token level for CADEC and MADE corpora respectively.

Conclusions: We present the system for extracting ADRs from texts describing the effects of taking drugs. The high level of obtained results proves the applicability of developed system for this task. A promising future research direction is

compare ADRs from systematic reviews and consumer social media posts in Russian.

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P040-T | Addressing medical coding of free-text clinical records in English with deep learning

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Background: Electronic healthcare records (EHR) are the central data sources that support clinical research and regulatory functions like billing. In these records, much data stored in the free-form text that are not otherwise translated into structured form. The standardization of medical terminology is critical for optimal communication and treatment across multiple healthcare systems. Coding of diseases and causes of death may be achieved by manually assigning a code. Vocabularies of terms used in coding are numerous including the International Classification of Diseases (ICD) and the Unified Medical Language System (UMLS). Therefore, it is highly beneficial to be able to map free-form text into an appropriate code automatically.

Material and methods: We explore an encoder-decoder with Long Short-Term Memory units directly tailored to the task of coding clinical texts. The idea behind this model is to map the input sequence to a fixed-sized vector, more precisely, some semantic representation of this input, and then unroll this representation in the target sequence of medical concepts.

Results: To evaluate the quality of our model, we adopted a standard benchmark from CLEF eHealth 2017 challenge. This benchmark consists of 28 163 texts from death certificates mapped to ICD-10 codes. Death certificates are standardized documents filled by physicians to report the death of a patient. Our model demonstrates state-of-the-art results on this corpus achieving an F-measure of 85% [1].

Conclusions: The proposed architecture demonstrates the ability to identify a sequence of medical concepts expressed free-form language of medical records. A promising future research direction could be focused on embedding code descriptions and codes into a latent space using UMLS structure.

Funding: This work was supported by the RSF grant no. 18-11-00284.

[1] Miftakhutdinov and Tutubalina. KFU at CLEF eHealth 2017 Task 1: ICD-10 Coding of English Death Certificates with Recurrent Neural Networks. CEUR Workshop Proceedings, 2017.

P041-T | Time budget of sleep analysis as welfare indicator using machine learning

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Background: Sleep loss in humans has been shown to be strongly correlated to physical and psychological well-being. This suggests evaluation of sleep patterns can be an indicator of animal welfare. In a project of the University of Salford and the Animal Science Center of Universidade Federal de Ouro Preto in Brazil, animal scientists collected video footage over eight consecutive months of several dogs living in the Animal Science Center in Brazil with the aim to analyze the sleeping patterns and look for correlations with welfare. The dataset contained 2.1 TB of data with 13 668 videos—analyzing it manually is a practically infeasible and error-prone task.

Materials and methods: To provide a solution, we developed a machine learning based approach to recognize sleeping behavior automatically. Several architectures of end-to-end neural network models for detection of sleeping and awake dogs were explored. Another approach was based on pose recognition by CNN model with heuristic post processing algorithm. In order to reduce the required amount of dataset every used neural network was trained using transfer learning approach.

Results: The use of hybrid models, including machine learning, classical approaches to image processing and heuristic algorithms, has led to a system for classification of asleep/awake dog with accuracy of 89% (out of 6000 frames, 5340 were correctly classified). These can be further improved by refining the models.

Conclusion: Machine learning tools have been shown useful for evaluation of animal welfare indicators. We envision such tools to be also useful in veterinary informatics. Further development of reusable and generic models for behavior analysis has the potential for impacting the welfare of companion, farm and zoo animals, which is a problem of increasing interest for the modern society.

P042-T | Prediction of drug induced mitochondrial dysfunction using QSAR and docking

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Mitochondria are the cellular components that generate 95% of the energy needed for a cell to remain viable. Our knowledge of how mitochondria function in the cell has expanded recently and it is now clear that mitochondria participate in nearly all aspects of cell function, affecting processes including cancer, inflammation, metabolic signalling and cell death. Given the key role that mitochondria play in metabolism and cell survival, it is of no surprise that they represent an attractive target for many antibiotics, fungicides, and many common poorly treated diseases such as metabolic disorders, neurodegenerative disorders, cardiovascular diseases and cancer.

Mitochondrial dysfunction as a result of drug-induced toxicities pose a major problem for the Pharmaceutical Industry, leading to drug attrition due to tissue toxicities such as drug-induced liver injury. Here, we have focused on the measurement of drug binding to certain members of the enzyme families in electron transport chain in mitochondria. We have then used molecular docking studies and QSAR to predict the electron transport chain inhibition by the drugs that can lead to mitochondrial dysfunction. We have focused on Complex II and Complex III inhibition by the drugs. The IC50 values were obtained using Succinate-cytochrome c reductase (SCR) activity assay for 15 drugs and literature IC50 values for additional compounds were obtained where the same methodology as ours had been used. The dataset (n = 35) was divided into test and training set and a Quantitative Structure-Activity Relationships model was obtained using the training set. The model showed a good predictive ability for the external test set according to the criteria proposed by Golbraikh and Tropsha (2002) for models to be considered acceptable including R^2 of 0.76 for the test set compounds. The docking results also indicated a significant correlation between log IC50 and the docking scores from MOE software.

P043-T | Changes in the level of phosphates, production of nitric oxide and pH levels of rats blood after spinal cord injury

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The search for new approaches to treatment of spinal cord injury (SCI) should take into account the multifactorial nature of metabolic disorders in SCI using new methods to obtain information about the molecular mechanisms. Therefore we investigated metabolic changes in the blood to assess possible shifts in redox system on a model of dosed SCI in rats. It was measured the level of organic and inorganic phosphorus compounds, pH level of blood by 31P NMR-spectroscopy and nitric oxide (NO) production by EPR-spectroscopy.

It was found increase the content of inorganic phosphates, phospholipids and monophosphates, as well as a decrease in rat's blood pH 3 days after SCI. There were also an increase in the amount of 2,3-diphosphoglycerate in red blood cells, which is one of the ways to increase oxygen supply to tissues under hypoxia, affecting the affinity of hemoglobin to oxygen. It was also found the increased NO production in damaged spinal cord tissue, blood, liver and heart. It is known that hypoxia induced synthesis of vasoactive metabolites, such as NO, increased the flow of blood oxygen to the tissues due to vasodilatation. Thus, the information obtained by NMR and EPR-spectroscopy makes it possible to determine the metabolic parameters that characterize the state of microcirculation and vasomotor reactivity, as well as the degree of tissue hypoxia and changes of acid-base balance in the blood with SCI. Activation of the NO production system and the detected increase in 2,3-diphosphoglycerate after SCI indicate compensatory-adaptive changes.

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P044-T | Antioxidant properties of the mixed product from juice and extract of *Abies sibirica* Ledeb

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The problems of regulating oxidative stress are in the spotlight of many researchers in various fields since a change in the oxidation-reduction processes in the body leads to serious health problems. Antioxidants are used for the regulation of redox processes, among which the most important are natural products, including vegetable origin. Fir needles are used in popular medicine, have a wide range of therapeutic properties, contain 3%-3.5% essential oil, 0.3% vitamin C. The aim of the work was to investigate the antioxidant properties of the mixed product obtained from the juice and extract of fir needles by its ability to interact with free radicals.

Mixed product (further product I) was obtained from the juice of cold pressed fir needles and its extract in proportion: juice 35%, extract 35%, emulsifier 0.01%, and distilled water (up to 100%). The extract was obtained by maceration with 3% ethanol solution. The antiradical properties of the product I in relation to AAPH free radicals were investigated by the chemiluminescence method on the chemiluminometer "Lum-100" (DISoft LLC, Russia). Flavonoid quercetin as an active antioxidant was determined by spectrophotometric method.

It is shown that when 10 μL of an undiluted product I and diluted 10 times (concentration of substances 0.5 and 0.05%, respectively) was added to the 1 mL of reaction mixture, complete stable suppression of chemiluminescence was observed, which indicates inactivation of all free radicals present in the system. For the product I, diluted 100 times, a gradual, linear recovery of chemiluminescence was observed, up to 35% of the initial level within 50 minutes, that is, the antioxidant properties were less pronounced. Quercetin content in the product I was 26 g/L.

Thus, the study revealed the ability of the mixed product obtained from the juice and extract of Siberian fir needles to interact with free radicals.

P045-T | Antioxidant and membranotropic properties of extracts from *Aronia melanocarpa* (Rosaceae) berries

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Redox processes are controlled by regulatory systems that support balanced interaction of oxidation products formation and antioxidant factors. Disruption of this interaction, accompanied by the activation of free-radical processes and the accumulation of oxidation products, is considered as a universal mechanism of damage to biological membranes underlying a number of pathological processes. Therefore, the focus of many researchers is studies of antioxidants. The purpose was to study antioxidant and membranotropic properties of extracts from *Aronia melanocarpa* (Rosaceae) berries.

Aqueous extracts were obtained by maceration of crushed berries with distilled water, alcohol extracts—with 70% ethanol solution. Antiradical properties of extracts in relation to AAPH free radicals were studied by the chemiluminescence method on the chemiluminometer "Lum-100" (DISoft LLC, Russia). Extracts were used in concentrations of substances: aqueous 1, 0.1, 0.01 mg/L, alcohol extracts 0.1, 0.01, 0.001 mg/L. The membranotropic activity of extracts was studied under conditions of osmotic and oxidative hemolysis of erythrocytes obtained from Sprague Dawley rats.

It was shown that the aqueous extract at a concentration of 1 mg/L and alcohol at a concentration of 0.1 mg/L completely inactivate free radicals, showing the maximum antioxidant effect. When incubating erythrocytes with aqueous extract in concentrations of 10-0.01 mg/L for 5 minutes, a decrease in osmotic hemolysis by 17%-27% was observed (maximum at a concentration of 0.1 mg/L), while incubating with an alcohol extract there was a decrease by 18%-35% (maximum at 0.01 mg/L). The maximum decrease in oxidative hemolysis by 80 and 50%, respectively, was observed when the concentration of the aqueous extract was 1, the alcohol extract was 0.01 mg/L.

Thus, the ability of *Aronia melanocarpa* berries extracts to interact with free radicals and exert a protective effect on erythrocyte membranes under osmotic stress and free radical-induced processes because of peroxide exposure was revealed.

P046-T | Effect of stress ulcerogenesis on the processes of oxidative modification of proteins in the blood serum of laboratory rats

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Background: Free radical oxidation and destruction of proteins is one of the early indicators of tissue damage, which justifies the need to study the dynamics of the formation of products of oxidative modification of proteins (OMP) in various pathological conditions of the body, including under the influence of various experimental models of stress. The aim of the work is to study the effect of stress ulcerogenesis on the processes of OMP in the blood serum of laboratory rats.

Material and methods: 16 male Wistar rats (180-220 g) were divided into intact and experimental groups of 8 animals. The experimental group was subjected to stress ulcerogenesis for 60 minutes according to the official experimental protocol. The content of OMP products at wavelengths (λ) 356, 370, 430 and 530 nm in the serum of all groups was studied by spectrophotometric method E. Dubinina and co-authors.

Results and conclusion: After exposure to stress ulcerogenesis, the level of OMP products in the blood serum of laboratory rats significantly decreased compared to the control group. The level of aldehydes and ketones of neutral character $\lambda = 356$ and $\lambda = 370$ nm relative to the control decreased by 23%, and the alkaline character at $\lambda = 430$

and λ = 530 nm—decreased by almost 3 times. The results obtained may be related to oxidation to carboxyl chemical groups of OMP products contained in the intact body after exposure to stress ulcerogenesis as a result of intensification of oxidative stress processes. This causes a decrease in the percentage of products registered by this method.

Thus, the level of OMP products under the influence of stress ulcerogenesis is significantly reduced, which indicates the intensification of oxidative processes and depletion under their influence of adaptive capacity of the organism in animals of the experimental group.

P047-T | Effect of hyperinsulinemia on oxidative modification of proteins in the nervous tissue of laboratory rats

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Background: Oxidative modification of proteins (OMP) is considered as a factor in the pathogenesis of nervous tissue in many diseases. Information about OMP in the nervous tissue during hyperinsulinemia contradictory.

The aim of the work is to study the effects of hyperinsulinemia on OMP products in the nervous tissue of laboratory rats.

Materials and methods: The material for the experiment was the nervous tissue homogenate of 32 laboratory male Wistar rats weighing 220-240 g. Laboratory rats were divided into 4 groups of 8 animals. The intact group was administered saline solution, group I-insulin 1 time per day; group II-insulin 1 time per day for 2 days; group III—insulin 1 time per day for 3 days. Insulin was administered subcutaneously at 3.5 Units. The presence of hypoglycemic coma was determined by the appearance of seizures. In all groups, the level of OMP products was determined at wavelengths of 356, 370, 430 and 530 nm by the method based on the formation of 2,4-dinitrophenylhydrazine derivatives.

Results and conclusion: It was found that hyperinsulinemia leads to a significant decrease in the nervous tissue homogenate of laboratory rats compared to the intact group of the content of OMP products at all wavelengths of registration ($P \leq 0.01$): in group I by 34.2%, in group II—by 37.2%, and in group III—by 38.8%. This indicates a decrease in the processes of free radical oxidation in the short-term hyperinsulinemia. Our data may be related with additional oxidation of OMP products in nervous tissue due to increased ROS when exposed to insulin and the formation of compounds containing carboxyl groups not defined as OMP products. For a more unambiguous conclusion, it is necessary to study the effect of hyperinsulinemia on the content of OMP products in hyperinsulinemia using fluorescent technology.

P048-T | Effect of hyperinsulinemia on oxidative modification of proteins in blood serum of laboratory rats

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Background: Hyperinsulinemia is a type of metabolic disorder that manifests itself both independently and as a concomitant to other diseases. Artificial hyperinsulinemia occurs due to inadequate insulin therapy in patients with insulin-dependent form of diabetes and used as a method of treatment of schizophrenia. Information on the processes of oxidative modification of proteins (OMP) in hyperinsulinemia contradictory. The aim of the work was to study the effect of artificially induced hyperinsulinemia on the OMP of blood serum in laboratory rats.

Material and methods: The experiments performed on blood serum of 32 male Wistar rats weighing 220-240 g, which divided into 4 groups of 8 animals. The intact group was administered saline solution; group I—insulin 1 time per day; group II—insulin 1 time per day for 2 days; group III—insulin 1 time per day for 3 days. Insulin was injected subcutaneously 3.5 Units each case. The appearance of seizures indicated the presence of hypoglycemic coma. In rats of all groups, the level of OMP products at wavelengths of 356, 370, 430 and 530 nm was determined by the method based on the formation of 2,4-dinitrophenylhydrazine derivatives.

Results and conclusion: It was found that the state of experimental hyperinsulinemia leads to a significant decrease in the level of OMP in the serum all experimental groups of rats compared with intact group. It is shown that the effect of insulin shock causes a decrease of almost an order of magnitude of the content of OMP products in animals of group III. Thus, artificially induced hyperinsulinemia in the short term does not lead to an increase in OMP and even contributes to its reduction. This method can be safe for use in medicine for short-term use.

P049-T | Protective effect of nitrite in brain ischemia/reperfusion via modulation of mitochondrial respiration

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Nitrite, once considered an inert metabolic endpoint of nitric oxide (·NO), has more recently emerged as a metabolic precursor of ·NO in vivo. This alternative source of ·NO may play a critical role in the brain under emergency conditions such as ischemia, when enzymatic ·NO production is hindered due to lack of oxygen. Evidence shows that nitrite is protective in situations of ischemia/reperfusion and appears to be beneficial in aging and neurodegeneration. Most relevantly, nitrite concentration in vivo can be modulated by diet through the ingestion of nitrate rich foods, being generally associated with increased longevity and lower incidence of cardiovascular disease.

One putative target for nitrite's protective bioactivity in ischemia is through modulation of mitochondrial respiration. Here we used high-resolution respirometry to determine the effects of nitrite on brain tissue respiration in conditions of ischemia/ reperfusion. We applied an in vitro ischemia/reperfusion protocol to permeabilized rat hippocampal tissue and determined the differences of complex I supported respiration in the presence and absence of nitrite. While under control (no nitrite) conditions, a significant increase in respiratory rate is observed upon re-oxygenation ("oxidative burst"), in the presence of nitrite (10 μmol/L), this burst is abolished. This inhibition may prevent the increased production of reactive oxygen species associated with this oxidative burst and may be one of the mechanisms through which nitrite is protective during brain ischemia.

Future studies are focused on confirming nitrite reduction to ·NO as a requirement for the inhibition of the oxidative burst as well as pin-pointing the site o nitrite/·NO bioactivity within the mitochondrial respiratory chain. Furthermore, we will explore if ascorbate can potentiate the protective effects of nitrite under ischemia/reperfusion conditions in brain tissue.

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P051-T | Iron accumulation in tissues in rat under gravitational unloading

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During space flight, a change in iron metabolism occurs. An excess of iron can lead to the formation of reactive oxygen species and induce ferroptosis. One method for studying the effects of iron accumulation in tissues and blood is the electron paramagnetic resonance spectroscopy method. The purpose of this work was to study the effect of gravitational unloading on the content of iron oxides in the tissues of the body, to estimate the parameters of the signals of electron magnetic resonance (EMR), to establish their source. Experiments were performed on nonlinear rats (n = 7), gravitational unloading was modeled by the method of antiorthostatic hypokinesia. In the tissues of the heart, lungs, liver and muscles, as well as in some blood samples, EMR signals were identified depending on the orientation. A comprehensive analysis of the characteristics of the EMR signals allowed us to determine the source of the signals in the form of crystalline iron oxides in magnetite or ferrihydrite (in crystalline ferritin basic) forms. Three types of signals were identified, corresponding to different spatial forms of accumulation of biogenic magnetite and ferritin. There were no similar signals in the tissues of the control group of animals. Thus, under conditions of gravitational unloading, there is an abnormal accumulation of aggregated forms of iron in the tissues of the lung, heart, liver, muscles, which indicates a change in iron metabolism and an abnormal accumulation of iron in the tissues and blood of rats. Effects on iron metabolism may be mediated by changes in hepatic hepcidin expression.

The reported study was funded by RFBR according to the research project № 19-04-01067.

P052-T | Redox state of adipose tissue and gastric cancer: connection with the body mass index and distance from the tumor

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Background: Excess body weight is causally linked to an increased risk of different cancer types, including

gastric (stomach) cancer (SC) but the mechanisms underlying this association are practically unknown. We investigate redox state of adipose tissues of patients with SC.

Material and methods: Superoxide (SO) generation rate, activity of complex I in electron transport chain of mitochondria by electron paramagnetic resonance (EPR), activity of matrix metalloproteinase (MMP-2 and 9) by gel zymography of adipose tissues (AT) of patients in the AT adjacent to tumor (ATAT) and at a distance of 3 cm (ATD) to follow the connection of the AT redox state with the microenvironment indicators and SC metastasis (HIF-1alpha, CD68, Plin5, M category) are measured according with the Helsinki Declaration of 1964 on AT of SC patients at disease stages II-IV (45 patients, 24 male, 21 female, 64.0 y.o), control AT obtained after liposuction (6 male, 5 female).

Results and conclusion: Defects in the mechanism of oxidative phosphorylation increase the SO generation rate, activity of MMP-2 and 9 in ATAT and ATD, activate the redox-dependent factors of tumor (HIF-1alpha, CD68, Plin5). High levels of SO generation and gelatinase activity in ATAT correlate with distant metastasis. Dysfunctional AT serves as a modifier of the microenvironment of gastric cancer facilitating its progression. The imbalance of the redox state of dysfunctional AT is coupled with obesity while the degree of its manifestation correlates with body mass index (BMI). The results could help to determine the new targets and prognostic markers for cancer patients with the weight excess as well as for the obese patients, may have a prognostic value in the treatment of SC patients.

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P053-T | Homocysteine-thiolactone at age-dependent manner induces seizure-likes events in rat hippocampus

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Background: An increase in plasma homocysteine concentration during pregnancy (prenatal hyperhomocysteinemia, hHCy) leads to imbalance in prenatal and postnatal development of nervous system which underlies a high risk of developing of epilepsy, migraine and ischemic damage. Hyperhomocysteinemia induced oxidative stress may occur as a result of decreased expression and/or activity of key antioxidant enzymes. Our study concerned the estimation of the effects of homocysteine derivatives—homocysteine-thiolactone on the neuronal activity in rat hippocampus vivo.

Materials and methods: Wistar rats of three age groups P5-7, P10-15 and P35-60 (P0-day of birth) were used. Extracellular neuronal activities were recorded from hippocampus using 16-site linear silicon probes. D,L-homocysteine-thiolactone was administrated by intrahippocampal injection using glass pipette. Multiunit activity (MUA), local field potential (LFP) were detected and analysed using MATLAB environment. Differences were considered as statistically significant at $P < 0.05$ in at least four independent experiments. Data are presented as mean ± SD.

Results: The lowest dose of homocysteine-thiolactone used (0.03 mg/2 μL) produced seizures in 75% of immature rats. The spectral power of LFP increased up to 1331 ± 662%, frequency of MUA—up to 1155 ± 598% compared with basal neuronal activity in CA1 region of hippocampus. In P10 and P35 animals that 0.03 mg/2 μL of homocysteine-thiolactone increased only MUA frequency and SLE appeared only at 0.06 mg/2 μL (in 100% of animals). The spectral power of LFP increased up to 668 ± 79% and 254 ± 32%, frequency of MUA up to 358 ± 45% and 396 ± 126% compared to control in P10 and P35 animals correspondingly. The spectral analysis of LFP indicated that homocysteine-thiolactone increased the power of SLE in theta and alfa band frequency.

Conclusions: Our data suggest that hippocampal neurons of immature rats have higher sensitivity to homocysteine-thiolactone which may underlie a high risk of seizure appearance in postnatal life in case of maternal hHCY.

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P054-T | Hydrogen peroxide reduces excitatory actions of GABA at the immature GABAergic synapse

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Reactive oxygen species are powerful modulators of neuronal and synaptic functions. Here, we explored whether a hydrogen peroxide affects the excitatory action of GABA at the immature GABAergic synapses. With this aim, we recorded multiple unit responses evoked by synaptically released GABA during electrical stimulation in the presence of glutamate receptor antagonists using extracellular recordings from CA3 pyramidal cell layer of neonatal (postnatal days P5-6) rat hippocampal slices. In control conditions, synaptic stimulation of GABA receptors evoked bursts of action potentials in CA3

neurons with a peak frequency of 0.39 ± 0.04 spikes/10 ms. The following application of hydrogen peroxide at concentrations of 30-180 μmol/L suppressed the evoked GABA responses. In the presence of hydrogen peroxide, the peak of MUA responses decreased to 35 ± 6% of control level ($n = 5$; $P < 0.05$). Similarly, the MUA density calculated within 300 ms after the stimulus was reduced by hydrogen peroxide to 39 ± 11% of control. Thereby, the hydrogen peroxide reduces excitatory actions of GABA at the immature GABAergic synapses that could result from a reduction in the intracellular chloride concentration, the reduction in the transmitter release and/or sensitivity to GABA of the postsynaptic site, as well as from a decrease in neuronal excitability.

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P055-T | Antioxidant activity of biomineral complex of citrus pectin and L-ascorbic acid

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It is widely known that overproduction of oxygen-derived free radicals induces cell damage which causes the pathological processes in living organisms. Pectins produced from plants can be considered as antioxidants, capable of scavenging free radicals [1]. Our previous investigations show that pectin complexes with metals have biological activity such as antianemic activity [2].

In present study biomineral complex of citrus pectin contained macro- and microelements and L-ascorbic acid (BCA) was obtained. The antioxidant properties of BCA in concentration 1 and 0.25 mmol/L calculated on ascorbic acid were studied by chemiluminescence method. It was demonstrated that BCA in concentration 1 mmol/L possesses antioxidant activity and suppresses chemiluminescence reducing its intensity to 10%-15% of the original. Slight decrease of chemiluminescence intensity of BCA to 85%-90% is observed in concentration 0.25 mmol/L calculated on ascorbic acid followed by reverse raising after 2000s to 225%, i.e., prooxidant properties appeared.

0.25 mmol/L ascorbic acid shows strong antioxidant properties completely stopping chemiluminescence caused by free radicals formation in reaction system. However, after 220 s a sharp rise of chemiluminescence intensity to 180% of the original is observed. It was revealed that 1 mmol/L ascorbic acid suppresses chemiluminescence as well. This effect is also unstable, a sharp rise of chemiluminescence intensity to 300% of the original after 400 s takes place. It's related to prooxidant properties of ascorbic acid transformation products.

To conclude the antioxidant properties of ascorbic acid as a part of biomineral complex with pectin become slightly weaker in comparison with ascorbic acid as no full scavenging of free radicals occurs. However, appeared antioxidant activity is more stable than one of ascorbic acid. At the same time, prooxidant properties of BCA decrease significantly.

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P056-T | Nitric oxide bioactivity in the hippocampus in aging and neurodegeneration

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Alterations in nitric oxide (NO) bioactivity plays an important role in decay of synaptic and metabolic functions in brain aging and Alzheimer's disease (AD). In the hippocampus NO production is linked to the activation of glutamate receptors, in particular NDMAr. We have demonstrated that NO concentration dynamics within this structure varies between cell type and that activation of the glutamate: nNOS:NO signaling pathway, due to NO diffusion across several cell diameters, regulates neurometabolic and neurovascular coupling. Here we investigate the putative changes in nitrergic signaling in the hippocampus during aging as well as in the triple transgenic mouse model of AD (3xTgAD).

We monitored NO concentration dynamics in hippocampal slices using carbon fiber microelectrodes coupled to electrochemical techniques. We challenged hippocampal slices from NTg and 3xTgAD mice at 3 ages (3, 12 and 18 m) and observed a significant increase in peak NO in young aged 3xTgAD, proposed to be an early compensatory mechanism for decay in synaptic plasticity. We also found an age-dependent decline in NO peak concentrations accompanied by a widening of the signal in 3xTgAD mice. This decay was contradictory to the age-dependent increase in nNOS expression in the 3xTgAD hippocampus, suggesting a deviation in NO bioactivity towards production of reactive species such as peroxynitrite, confirmed by the increase in 3-NT immunostaining. We also found a decrease in oxygen consumption rate in hippocampal slices from 18-mo old mice. Using a customized high resolution respirometry protocol for intact hippocampal slices to evaluate mitochondrial oxidative

phosphorylation, we found an age-dependent comprise in respiratory sparing capacity.

Our data support the notion that changes in the redox environment during brain aging and neurodegeneration contribute to derailment in NO bioactivity in the brain, contributing to synaptic and bioenergetics crisis that undermines the two processes.

P058-T | Determination of the antioxidant potential of the leaves of *Acrocomia aculeata* (Jacq.) Lodd. ex Mart.

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Background: *Acrocomia aculeata* is a palm native from the Brazilian cerrado, which use is described for the treatment of various diseases. Thus, this study aimed to evaluate the chemical composition, antioxidant activity and toxicity of *A. aculeata* leaves.

Methods: Extracts with water (EA-Aa), ethanol (EE-Aa) and methanol (EM-Aa) were produced, in which the concentrations of phenolic compounds, flavonoids and tannins were determined by chromatography. The antioxidant activity of the extracts was determined by free radical scavenging generated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and by inhibition of hemolysis and lipid peroxidation induced by 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) in human erythrocytes and by 5-hydroxy-1,4-naphthalenedione (Juglone) in Caenorhabditis elegans induced with.

Results: The following compounds were identified: gallic acid, caffeic acid, ferulic acid, rutin and quercetin by LC-PDA analysis for all extracts, except the vanillic acid, found only in EA-Aa, and campesterol, stigmasterol, β-sitosterol, lupeol and lupeol acetate, obtained in EE-Aa e EM-Aa by GC-MS analysis, which have also shown higher amounts of phenolic compounds, flavonoids and tannins and free-radical scavenging potential (12.92 ± 0.32 e 13.28 ± 1.2 μg/mL) than the EA-Aa (117.10 ± 7.35 μg/mL), and similar to standard controls (ascorbic acid and butylated hydroxytoluene). However, in the lipid peroxidation induction assays, 500 and 1000 μg/mL EA-Aa was able to more efficiently protect erythrocytes from AAPH action, resulting in lower levels of hemolysis and MDA after 4 hours of exposure. In in vivo assays, the results confirm that 750 and 1000 μg/mL EA-Aa promoted greater C. elegans protection against Juglone-induced oxidative stress, indicating greater viability of the nematodes after ROS induction. In turn, toxicity was remarkably low in C. elegans, with LD50 values of 2000 μg/mL.

Conclusions: Together, the extracts of *A. aculeata* leaves presented low toxicity, relevant antioxidant potential, which is probably related to their rich chemical composition.

P059-T | Intense pulsed light on skin carcinogenesis: histological analysis

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Background: Intense pulsed light (IPL) is a non-laser high intensity light extensively used in dermatology and for esthetics purposes. This work intended to evaluate the effects of IPL in a mouse model of skin carcinogenesis.

Materials and methods: Procedures followed the European legislation (Directive 2010/63/EU). Sixteen ICR female DBA/2JRccHsd mice were randomly divided into two experimental groups: IPL-exposed ($n = 8$) and non-exposed ($n = 8$). The dorsal region of mice was shaved using a machine clipper. Animals were applied with the carcinogen 7,12-dimethylbenz[a] anthracene (DMBA; 2 mmol/L, single dose) and 12-O-tetradecanoylphorbol-13-acetate (TPA; 100 mmol/L, twice a week, for 22 weeks). IPL-exposed animals were applied with IPL (intensity of 2 J/cm^2, twice a week, for 22 weeks). At the sacrifice, skin samples were collected and processed for histological analysis.

Results and conclusions: A total of 74 pre-neoplastic and neoplastic skin lesions were observed: 28 in IPL-exposed group and 46 in non-exposed group ($P = 0.066$). Twenty lesions of the IPL-exposed mice and 38 lesions of non-exposed mice were classified as neoplastic epidermal lesions ($P = 0.018$).

Papilloma grade II was the most frequent neoplastic lesion observed in both experimental groups. The higher number of malignant lesions was observed in IPL-exposed group when compared with non-exposed group (three microinvasive squamous cell carcinoma in IPL-exposed animals vs one microinvasive squamous cell carcinoma in non-exposed animals). These results suggest that IPL may inhibit the skin carcinogenesis, but it seems to promote the malignant conversion.

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P060-T | Development of a curcumin derivative with hypoglycemic properties: Impact on oxidative stress and endothelial function in type 2 diabetes

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Background: The anti-inflammatory and antioxidant properties of curcumin may be determinant in the treatment of metabolic diseases, such as diabetes. Due to its strong intrinsic activity, a newly curcumin derivative has been synthesized to potentiate its effects. So, the aim of the present study was to evaluate the hypoglycemic effects of the derivative and its effects on endothelial function in type 2 diabetes.

Methods: For that purpose, 14 weeks Goto Kakizaki (GK) rats were treated for 2 weeks by s.c. injection with the same quantity (mmol) of curcumin (40 mg/kg/d) or the derivative (52.4 mg/kg/d). Two groups of 5 rats were used as control and vehicle. Fasting glycemia, insulin tolerance, body weight and caloric intake were evaluated during treatment. After sacrifice, aorta rings were prepared for cumulative concentration-response (CR) isometric relaxation curves to acetylcholine (ACh, 0.01 to 90 µmol/L) after precontraction with 10 µmol/L noradrenaline (NA) in the presence and absence of 100 µmol/L ascorbic acid or 250 µmol/L N^G-nitro-L-arginine methylester (L-NAME, selective inhibitor of endothelial nitric oxide synthase).

Results: Although no significant alterations were observed in caloric intake or body weight, curcumin appears to have a slight hypoglycemic effect, that was potentiated by the derivative. No significant differences were observed in the ACh-induced maximum relaxation between diabetic groups, but the treatment with the derivative significantly increased the maximum relaxation in the presence of ascorbic acid, revealing more sensitivity to antioxidants compared to GK controls and restoring normal response observed in the Wistar control group. L-NAME abolished the ACh vasorelaxant effect in all animal groups confirming NO involvement on vessel relaxation.

Conclusions: Taken together, these data suggest that the derivative potentiate the effects of curcumin, improving the fasting glucose and the endothelial function on type 2 diabetes, which is associated with increased activity of antioxidant enzymes.

P061-T | On the hunt for quality: Reactive oxygen species—Can we use them in our favor?

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Background: Worldwide, 10%-15% of couples face infertility issues, with male factor responsible for 50% of the cases. Also, the average success of assisted reproductive techniques (ART) remains at 32%. This is mainly due to the high heterogeneity of the ejaculates (containing both functional and non-functional gametes) and to the unsophisticated routine sperm analysis, which has limited predictive power, and has remained unchanged for decades. This highlights the need to identifying function-based sperm biomarkers that can be used to improve diagnostics and select the best gametes for ART. The established association between mitochondrial functionality and sperm quality, enhances the use of mitochondrial parameters, such as ROS (reactive oxygen species)

levels, as a tool to identify and segregate a more functional sperm subpopulation.

Material and methods: Human sperm samples were collected from adult males undergoing fertility treatment and were analysed in order to obtain data regarding sperm parameters, such as concentration, pH, motility and morphology. Sperm cells were then incubated with fluorescent probes against ROS. The cells were then analyzed through Flow Cytometry and the results were posteriorly correlated with the sperm parameters previously determined.

Results and conclusion: The obtained results show statistically significant correlations between ROS levels and sperm parameters. Despite the not so high correlations (values vary between $r = 0.2$ and 0.4), our results prove once again the association between mitochondrial functionality and sperm quality. This suggests a potential use of ROS as a biomarker for the selection of the best gametes. Furthermore, this opens up the possibility of performing further studies exploring more function-based sperm biomarkers within the selected population leading, ultimately, to the increase in ART success.

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P062-T | Inflammation in endothelial dysfunction associated with type 2 diabetes: Role of perivascular adipose tissue

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Background: Perivascular adipose tissue (PVAT) exerts vasodilatory and anti-inflammatory functions but it changes under pathologic conditions. Propagermanium (PG) has immune modulating activity and anti-inflammatory properties. This work aimed to study the therapeutic efficacy of PG in endothelial dysfunction associated with type 2 diabetes and its perivascular impact.

Material and methods: Two different animal models were evaluated: Goto-Kakizaki (GK) diabetic rats and control Wistar rats. GK rats were divided into four groups: (1) control group; (2) group treated with 50 mg/kg propagermanium; (3) group of GK rats fed a high fat diet (GKHFD) and (4) group of GKHFD treated with 50 mg/kg of propagermanium. The propagermanium was given orally for 3 months. Several in vivo parameters such as body weight, nasoanal length (for the Lee Index), lipid profile (total cholesterol and triglyceride systemic levels), fasting glucose levels, glucose tolerance and insulin sensitivity (through glucose and insulin tolerance tests, respectively). At the vascular level, endothelial dependent and independent vasorelaxation and contraction studies were performed.

Results: We found that oral administration of propagermanium didn't interfere with animal weight and didn't change Lee's index, however, it improved fasting glucose levels and insulin resistance, it didn't change total cholesterol and triglyceride levels, it didn't have a significant effect on the intraperitoneal glucose tolerance test, improved insulin sensitivity and endothelial dysfunction, and recovered the anti-contractile effect of perivascular adipose tissue.

Conclusions: The presence of propagermanium improved endothelial dysfunction and recovered the vasodilating phenotype of perivascular adipose tissue probably due to its anti-inflammatory features. So, perivascular adipose tissue is involved in the regulation of endothelial function and appears as a potential therapeutic target for vascular dysfunction related to type 2 diabetes.

Funding sources: PTDC/BIM-MET/4447/2014; POCI-01-0145-FEDER-016784.

P063-T | Omentin as a new therapeutic potential to target perivascular adipose tissue dysfunction

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Background: Perivascular adipose tissue (PVAT) may locally influence the functioning of blood vessels and may lead to vascular complications associated with diabetes and obesity. The aim of this work was to study the impact of omentin-1 on endothelial function in a non-obese type 2 diabetes mellitus animal model, Goto-Kakizaki (GK) rats fed with or without high fat diet.

Material and methods: GK rats were divided into four groups: (1) control group; (2) group treated with omentin-1; (3) group of GK rats fed a high fat diet (GKHFD) and (4) group of GKHFD treated with omentin-1. Several in vivo parameters such as body weight, nasoanal length (for the Lee Index), lipid profile (total cholesterol and triglyceride systemic levels), fasting glucose levels, glucose tolerance and insulin sensitivity (through glucose and insulin tolerance tests, respectively). At the vascular level, endothelial dependent and independent vasorelaxation and contraction studies were performed.

Results: It was shown that the PVAT anti-contractile action found under physiological conditions is lost in type 2

diabetes, and partially recovered with omentin-1 administration. Furthermore, it was observed an improvement in various systemic and metabolic biochemical parameters of diabetic animals treated for one month with omentin, namely circulating triglyceride levels, fasting glycaemia and insulin tolerance.

Conclusions: With this work we concluded that omentin presents therapeutic potential in type 2 diabetes.

Fundingsources:PTDC/BIM-MET/4447/2014;POCI-01-0145-FEDER-016784.

P064-T | Vascular dysfunction in type 2 diabetes: Role of perivascular adipose tissue

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Background: Perivascular adipose tissue (PVAT) surrounds most large blood vessels and plays an important role in vascular homeostasis. The present study was conducted to investigate the contribution of PVAT to vascular dysfunction in a rat model of type 2 diabetes (GK) fed with a high-fat diet (HFD).

Material and methods: Aortic vascular reactivity was performed in GK, GK fed with HFD and compared with Wistar rats in the absence (PVAT−) or in the presence (PVAT+) of thoracic PVAT. We also measured vascular oxidative stress.

Results: Endothelium-dependent relaxation to acetylcholine, assessed by wire myography, was impaired in GK and GKHFD rats, not affected by NG-nitro-L-arginine methyl ester, an inhibitor of endothelial nitric oxide (NO) synthase, and improved by the antioxidant tempol, suggesting reduced NO bioavailability and increased oxidative stress. Furthermore, vascular superoxide and peroxynitrite production was increased in the vascular wall of diabetic rats. The presence of PVAT had an anti-contractile effect in response to phenylephrine in Wistar rats. In GK rats, the anti-contractile effects of PVAT were lost.

Conclusions: Our data suggest that this rat model of type 2 diabetes is associated with perivascular adipose dysfunction, oxidative stress and endothelial dysfunction.

Funding sources: PTDC/BIM-MET/4447/2014; POCI-01-0145-FEDER-016784.

P065-T | A hydrophilic carbon nanomaterial for biomedical applications: Modulation of cell redox state and neuroprotective effects

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Oxidative stress plays a key role in pathological aging processes, underlying many human chronic diseases, including degenerative brain diseases. Therefore, nontoxic nanomaterials with ability to preserve and/or to restore the physiological redox state of cells under oxidative stress conditions can provide a novel insight for therapeutic applications. To pursue this goal, we propose a new generation of hydrophilic carbon nanomaterials produced by an electrochemical approach developed by our group. In the present work, we report the relationship between the physical-chemical properties and bioactivity in the context of Alzheimer's disease of the carbon nanomaterial synthetized in citrate buffer. It was found that the carbon-based nanomaterial exhibits a structure dominated by sp^2 carbons in a non-order carbon network formed by small clusters (<2 nm) of a carbonaceous material with many hydroxyl groups. This nanomaterial has an outstanding electron-donating ability, as revealed by cyclic voltammetry, and negative surface charge, evaluated in terms of Zeta potential. Antioxidant assays showed that it exhibits a high radical scavenging activity against both DPPH and ABTS radicals, and a concentration-dependent capability to protect the mitochondrial lipids and intracellular thiol groups from oxidation promoted by tert-butyl hydroperoxide and hydroxyl radicals. Cell-based assays revealed that the hydrophilic carbon nanomaterial has ability to protect, at non-cytotoxic concentrations, the neuronal cells against oxidative damage and toxicity promoted by tert-butyl hydroperoxide and amyloid-$\beta_{1\text{-}42}$ peptide. These neuroprotective effects emerge from its ability to preserve the redox state of cells (GSH/GSSG ratio) and to decrease the rate of ROS generation. Since oxidative stress and amyloid-$\beta_{1\text{-}42}$ peptide toxicity are key players on the degenerative brain process underlying Alzheimer's disease, the present work opens further investigations for potential medical applications.

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P066-T | Neonatal focal epilepsy detection based on the optical intrinsic signal

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Neonatal epilepsy is a paroxysmal condition that occurs in premature and full-term newborns during the early postnatal period. More than half of the cases of neonatal epilepsy are focal epilepsy, which is characterized by the presence of a focus—the epileptogenic area of the cortex. However, detection of the foci of focal neonatal epilepsy is not trivial and requires the development of new techniques and approaches.

Previously, we have demonstrated the efficiency of the optical intrinsic signal (OIS) in the localization of the active cortical barrels. We have equally shown that in contrast to adults, in neonates the OIS is predominantly generated via changes of light scattering of the active neuronal tissue. We proposed that OIS could be efficient in the detection of epileptogenic regions in the developing brain. To test this hypothesis we initiated focal epileptic activity using 4AP in rat pups during their first postnatal month and recorded both the electrophysiological activity and the OIS. We suggested that OIS would be characterized by the earliest onset in the epileptogenic region thus the cross-correlation approaches were used to detect the position with the earliest OIS.

We have found that local application of the 4AP resulted in the epileptic activity associated with the OIS, which was characterized by the higher amplitude compared with the sensory driven OIS. Epileptic OIS propagated over the cortex following the EEG signs of the epileptic activity with a delay of 2.7 ± 1.7 seconds. Using cross correlation analysis both in mature and neonatal rats we localized foci of the pharmacologically evoked epileptic activity that was found in the close region to the 4AP injection site, confirming our hypothesis. Thus, the position of the epileptogenic foci in the developing nervous system can be efficiently detected by the OIS registration. This work was supported by RSF grant 16-15-10174.

P067-T | Vitamins group B restored the motor deficit of the rats with maternal hyperhomocysteinemia

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Background: Prenatal hyperhomocysteinemia (hHcy) is a disease that can be associated with a genetic mutation of the enzymes of methionine metabolism or deficiency in the vitamins group B which are essential cofactors of the enzymes activity during pregnancy.

The aim of this work was to study the effects of vitamins group B supplementation of dams on the muscle strength and motor coordination of the offspring with prenatal hHcy.

Material and methods: The experiments were carried out on the offspring (at P26) which were divided into 4 groups in accordance with the diet of dams: (1) the control group (n = 53 pups) from dams on the usual diet; (2) the homocysteine (Hcy) group (n = 65) from dams with hHCY; (3) the group B (n = 26) from dams received vitamins pyridoxine 10 mg/kg, folic acid 0.25 mg/kg and cobalamin 0.25 mg/kg; (4) the Hcy-B group (n = 26) from dams with hHCY received vitamins at the same concentration.

Results: The muscle strength was measured by the Paw Grip (PaGE) endurance test, were the time spent on the grid (before the fall) was recorded. The rats from Hcy group demonstrated the reduction of time spent at the grid (76 ± 8 vs 107 ± 7 s in control). This parameter was restored to the control level in rats from the Hcy-B group (110 ± 8 s). Motor coordination was evaluated using a rotarod test. A significant reduction in the time spent on the rotarod and distance were observed in the Hcy group compared with the control. Again treatment with vitamins restored both parameters to control values.

Conclusions: Introduction of folic acid, pyridoxine and cobalamin to the diet of females prevented the toxic effect of Hcy on the muscle strength and motor coordination of the offspring.

Funding: The work was supported by RSF (19-15-00174).

P068-T | Modulation of multisegmental responses in leg muscles during postural tasks

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The amplitude modulation of multi-segmental responses (MMRs) can reflect the result of the integration of sensory and motor information at the spinal level during postural and motor control. The aim of this study is to investigate effects of various postural tasks on alteration of characteristics of multi-segmental responses in healthy subjects. Six relevantly healthy individuals (23.2 ± 2 years) were examined under three experimental conditions: eyes open on a normal floor surface; eyes closed on a normal floor surface; and eyes open on a foam surface. At conditions transcutaneous electrical spinal cord stimulation was applied at the Th11-12 vertebra.

The MMRs were registrated by bipolar self-adhesive electrodes from the following muscles: rectus femoris (RF), medial hamstring (MH), tibialis anterior (TA), and soleus (SOL). Our results indicate that the modulation of MMRs amplitude was mainly observed in calf muscles. Particularly, we observed suppression in amplitude of MMR in TA and SOL ($P < 0.05$), but not in RF and MH ($P = 0.05$) with more complex tasks (eyes closed and foam surface) Consequently, MMRs modulation under various postural tasks can be related on presynaptic inhibitory reflexes under alteration of afferent input. These results can provide essential information concerning postural control. The reported study was funded by RFBR according to the research project №18-315-00263.

P069-T | Empowering women: In vitro safety and efficacy of cationic surfactants as active principles for potential vaginal contraceptives

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Background: The rate of unwanted pregnancies is especially high in developing regions, mainly due to limited access to health services and poor women empowerment. Thus, there's a need for developing effective and safe vaginal contraceptives that meet the personal and cultural needs of these women.

Benzalkonium chloride (BKC) and myristalkonium chloride (MKC) have been used in contraceptive formulations despite the few reports targeting their efficiency and tolerance. Thus, this pre-clinical in vitro study aimed to address both the efficacy and safety of BKC and MKC using human sperm samples and HeLa cells to mimic the vaginal/cervical epithelium, and to compare it with nonoxynol-9 (N9), a surfactant proved toxic to the human cervical epithelium.

Material/methods: Twelve normozoospermic samples were assessed for motility, viability and acrosome status after exposure to an untreated control, N9 and different concentrations of BKC and MKC present in available formulations for 0 and 10 minutes. While sperm motility was evaluated by both phase-contrast microscopy and the Sander-Cramer test, viability was monitored by the eosin assay and the acrosomal status by the PSA-FITC marker. HeLa cells viability and metabolic activity (n = 6) were evaluated by the Trypan-blue and MTT assays after 0 and 1 hour of incubation in the same experimental conditions.

Results: Significant decreases in all sperm parameters were observed when compared to the untreated control, confirming the contraceptive efficacy of the compounds at the tested concentrations ($P < 0.001$). As for HeLa cells, both assays showed an extremely high cytotoxicity at these concentrations ($P < 0.01$). No differences were detected when BKC and MKC were compared with N9 in both cell types.

Conclusions: Even though these compounds are effective spermicides, they have the potential to damage the cervical epithelium. Testing lower concentrations is required to find a concentration that retains the spermicidal effect without causing harm to women.

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P070-T | Micronutrients and TCRαβ CD8αα intraepithelial lymphocytes in colorectal cancer

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Colorectal cancer is the second leading cause of cancer mortality in more developed countries. Age, lifestyle and chronic inflammation are risk factors to develop colorectal cancer.

Intestine provides absorption of nutrients and water, but is exposed to massive environmental challenges. Intraepithelial lymphocyte (IEL) subsets such as TCRαβ CD8αα IELs (CD8αα) live in intimate crosstalk with epithelium and play immunoregulatory functions in intestinal mucosa. Whether this immune population plays critical roles in tumour immunosuppression is still unknown. Interestingly, our preliminary data show that, in murine models, T cell-dependent retinoic acid signaling disruption compromises CD8αα IEL intestinal population and is associated with an increased colorectal tumour size and progression. Thus, indicating that retinoic acid signaling controls CD8αα IELs and micronutrient-regulated CD8αα IELs can potentially play a role in carcinogenesis. We believe that the cutting edge of our work will provide insights into colon cancer immune microenvironment, paving the way for new prognostic biomarkers and potential targets for innovative treatments such as immunotherapy, which is still emerging for colorectal cancer.

P071-T | Computational approaches to neutrophil transcriptome analysis

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Background: Neutrophils contribute to disease pathology in inflammatory diseases including rheumatoid arthritis (RA), lupus and vasculitis. Using RNA-seq we have (a) replicated the complex RA gene expression profiles observed in vivo by using combinations of agonists in vitro; (b) identified RA blood neutrophil gene biomarkers that stratify RA patients into responders and non-responders to TNFi therapy; (c) characterised a subset of neutrophils in RA (low-density granulocytes, LDGs), which may be novel disease regulators arising from developmental plasticity. This aim of this work is to use computational approaches to construct a model of gene expression in RA neutrophils, which can predict regulators of inflammation in sub-groups of patients (disease responders/non-responders; early/severe disease) and correlate gene expression profiles with clinical demographics and markers of disease activity.

Methods: RNA-seq datasets from RA neutrophils (n = 15 early RA; n = 23 severe RA) were mapped to the human genome (hg38) using TopHat and annotated using Cufflinks. Computational analysis was carried out using ARACNE2, GALGO and GSEA. Gene networks were reconstructed using Cytoscape. Functional annotation was carried out using Ingenuity and DAVID.

Results: ARACNE2 identified the main co-regulated networks of genes in RA neutrophils as: transcription, translation and post-translational modification of proteins; protein transport and localisation; response to activation of an immune cell receptor; innate immune response to interferon-alpha. GALGO identified networks of genes which predict clinical characteristics, including disease activity score (DAS28), ESR and CRP titres, and response to TNF inhibitor therapy. GSEA identified enrichment of genes on chromosome 6 in RA patients compared to healthy controls. Enriched genes regulate chromatin assembly, antigen presentation, apoptosis and activation of IκB/NFκB.

Conclusions: Computational modelling of neutrophil gene expression identified complex networks of co-regulated genes which correlate with altered phenotype in RA. The model will be developed, refined and tested throughout the duration of this project.

Funding: Versus Arthritis.

P072-T | Endotoxemia accelerates atherogenic monocyte recruitment through NET-resident H2A

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Background: Acute infection is a well-established risk factor of destabilization of pre-existing atherosclerotic lesions. However, the nature of the underlying processes remains unclear. Of note, epidemiologic studies show that endotoxemia results in heightened lesion development as well as acceleration of atheroprogression. LPS is a potent activator of circulating immune cells including neutrophils, which foster inflammation through expelled chromatin (NETs).

Material and methods: To investigate the role of endotoxemia-induced NET-formation in atherosclerosis we studied arterial monocyte adhesion by intravital microscopy of the carotid artery. Furthermore, mechanisms of monocyte adhesion to NETs were studied in vitro adhesion assays and by atomic force microscopy.

Results: In endotoxemia neutrophils release NETs decorated with different granule proteins and as well as histones. Our data show, that NET-resident H2A causes charge dependent monocyte adhesion to NETs and accelerates atherosclerosis. If H2A is blocked, arterial monocytes adhere less and lesion formation is reduced.

Conclusions: This study provides a mechanistic link between NETs and monocyte adhesion at the site of developing atherosclerotic lesions. By combining a mouse model of early atherosclerosis and in vitro studies, we identified that endotoxemia-driven NET formation accelerates atherosclerosis through increased monocyte adhesion at sites of atherosclerotic lesions, which can be limited by blocking NET-resident H2A.

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P074-T | Neutrophil proteo-interactome: Tracking neutrophil-derived messages in health and disease

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Background: Cross-talk between cells is crucial for multicellular organisms, especially within immune system. The initiation of the message cascade between immune cells during

the onset of inflammation is mostly mediated by neutrophils. Current approaches to study neutrophil communications tend to mimic the cell environment in vitro, as well as focus on narrow, pre-defined number of messages and just one type of cell-receiver. In this study we were aiming to develop a proteomics-based assay that allows to overcome these limitations and to track the whole spectrum of neutrophil-derived proteins in the entire blood.

Methods: The assay is based on SILAC labelling (stable isotope labelling with amino acids in cell culture). The proteins are labelled upon differentiation of neutrophil precursor HoxB8 cell line into neutrophils (HoxB8-PMN). The labelled proteins are released following stimulation, received by co-incubated cells, and can be distinguished in the proteome of the latter ones using mass spectrometry. The cells are separated after co-incubation using fluorescence assisted cell sorting.

Results: To confirm feasibility of the proposed approach we have performed a pilot experiment with RAW264.7 cells as receivers and PMA (phorbol myristate acetate) as a stimulus for HoxB8-PMN. In total 404 labelled proteins have been quantified in RAW264.7 cells sorted after co-incubation with HoxB8-PMN (20% of all quantified proteins in RAW264.7). These proteins are frequently released to extracellular space according to pathway analysis and included known neutrophil-released molecules (MIF, HSPs, histones, annexins, S100A8/9, etc.) that supports their neutrophil origin. The effect of neutrophils on macrophages was also apparent in principle component analysis.

Conclusions: The pilot experiment indicated that neutrophils have a great effect on macrophages in inflammation and provided support for the assay principle. The next step in the assay development would be to detect labelled HoxB8-PMN derived proteins in other immune cells from the whole unprocessed mouse blood.

P075-T | Is type 1 diabetes a NET-driven whole-pancreas disease?

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Human type 1 diabetes (T1D) is a predictable autoimmune disease characterized by the presence of islet-specific autoantibodies that can be detected years before the clinical onset, a stage recognized as pre-symptomatic T1D. Our group demonstrated that not only adaptive immunity but also neutrophils are abnormal in T1D, suggesting that these cells might play a role in T1D development. Although often neglected, according to new recent data, not only endocrine irregularities, but also exocrine subclinical abnormalities exist in the pancreas of T1D patients, already in the pre-symptomatic stage. It remains to be elucidated the underlying pathological mechanism leading to this whole pancreas damage in T1D. Immunofluorescence analysis of neutrophils was performed on pancreas sections from nPOD and DiViD cohorts and histological reports from the nPOD datashare were collected. It was observed that a higher number of neutrophils is present in the pancreas of T1D subjects as compared to non-diabetic organ donors. A fraction of pancreas-infiltrating neutrophils releases Neutrophil Extracellular Traps (NETs), pathogenic component of several autoinflammatory disorders. Despite T1D is a β-cell-specific disease, neutrophils and NETs were found infiltrating both endocrine and exocrine pancreas. Tissue-residing NETs in the exocrine pancreas, are known to contribute to plug formation, duct obstruction and pancreatitis. We therefore analyze data collected from the nPOD datashare and found a higher presence of histological signs of pancreatitis in both pre-symptomatic and symptomatic T1D subjects as compared to non-diabetic organ donors. Convincing data support the hypothesis that T1D is a disease of the whole pancreas, in which the loss of functional beta-cell mass is most clinically apparent. Our results demonstrate that both NETs and signs of pancreatitis are present in the pancreas of pre-symptomatic and symptomatic T1D subjects. We therefore hypothesize that neutrophil infiltration and NET formation might play a key role in the development of T1D exocrine-endocrine pancreatopathy.

P076-T | Neutrophil-borne proteinase 3 confers plaque vulnerability in late atherosclerosis

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Background: Cardiovascular diseases (CVD) are the leading mortality cause of industrialized countries, representing 31% of deaths worldwide. The rupture of advanced atherosclerosis lesions, one of the pathological conditions encompassed among CVD, can lead to myocardial infarction and ischemic stroke. Neutrophil serine proteases (NSPs); Cathepsin G (CG), Neutrophil elastase (NE) and Proteinase 3 (PR3) have been located in murine and human atherosclerosis lesions. Despite of the efforts to delve into the involvement of NSPs

in atherosclerosis, their implication in plaque instability remains unsettled.

Material and methods: Atherosclerosis was induced in Apoe-deficient mice by high fat diet feeding during 16 weeks. The disease was evaluated in three groups of mice lacking NSPs; CG, NE, and PR3/NE on an Apoe-deficient background, and compared to control mice (Apoe-deficient). Lipid accumulation (Oil red O), Collagen (Picrosirius red), plaque size, fibrous cap thickness and necrotic core (Hematoxylin/Eosin) were studied through histology. The cellularity of the lesions (macrophages, neutrophils, smooth muscle cells) was assessed by immunohistochemistry. The number of dead cells was quantified by TUNEL.

Results: The evaluation of the plaque size in the aortic roots did not show important differences in size, lipid, macrophage or neutrophil content among the NSPs deficient and control mice. However, PR3/NE deficient mice presented signs of decreased plaque instability; higher fibrous cap thickness, smooth muscle cell and collagen content, but lower necrotic core and number of dead cells as compared to control mice. Interestingly, the plaque characterization of NE deficient mice did not contrast with the control group, indicating that the differences found in PR3/NE deficient mice are due to PR3.

Conclusions: These results demonstrate that PR3 increases the vulnerability of advanced atherosclerosis lesions and lay the foundation for future studies to test therapeutic applicability.

P077-T | Dihomo-gamma-linolenic acid alters the proteomic releasate profile and reduces platelet reactivity

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Background: The fatty acid dihomo-gamma-linolenic acid (DGLA) reduces platelet aggregation directly and following dietary intake, potentially by altering eicosanoid production. Some of these eicosanoids are produced via cyclooxygenase-1, the target of aspirin. Here, we have investigated the acute effects of DGLA on platelets in both the presence and absence of aspirin.

Material and methods: Washed platelets were incubated with aspirin (30 μmol/L) or vehicle for 30 minutes, then with DGLA (10, 20 or 50 μmol/L) or vehicle for a further 3 minutes, and then stimulated with thrombin (1 U/mL). Releasates were collected and protein composition measured by label-free liquid chromatography-tandem mass spectrometry with analysis by Perseus and Ingenuity Pathway Analysis software. In separate studies, light transmission aggregometry was used to follow responses of aspirin-treated platelet-rich plasma to TRAP-6 amide (3 μmol/L) in the presence of DGLA (1 mmol/L) or vehicle. Platelet adhesion to fibrinogen-coated coverslips was visualised using immunofluorescence.

Results: On its own, DGLA altered release of 30 proteins out of 503 detected in releasates (n = 3). In the presence of aspirin, DGLA altered release of 138 proteins including reductions in adhesion proteins (vWF, THBS1) and immune mediators (CXCL7, C5). Pathway analysis predicted decreased platelet reactivity (aggregation, adhesion, spreading), inflammatory responses and increased vascular repair. DGLA inhibited platelet aggregation (72 ± 2% to 28 ± 13%) and potentiated aspirin-mediated inhibition (64 ± 4% to 6 ± 2%) (n = 5), as well as decreased platelet adherence (PBS, 14 ± 1 platelets per field of view; DGLA, 5 ± 1) and average spread area (PBS, 44 ± 4 μm^2; DGLA, 13 ± 3 μm^2) (n = 5).

Conclusions: We have shown that DGLA alters protein release from platelets, reduces platelet reactivity and might also influence platelet-dependent inflammation and tissue regeneration. Some of those effects were enhanced in the presence of aspirin, suggesting that aspirin and DGLA could act through different eicosanoid pathways. This supports the possibility of a dual treatment approach for diseases linked to platelet activation, such as thrombosis.

P078-T | Naturally occurring COOH-terminal, but not NH_2-terminal fragments of serum amyloid A1 (SAA1) do not exert direct chemotactic activity, but retain FPR2-binding and potentiating activity in neutrophil migration to CXCL8 and in monocyte migration to CCL3

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Background: SAA1 is a major acute phase protein in humans, induced to high levels by physical insults, including inflammation and infection. SAA and its NH_2-terminal part have been studied extensively in the context of amyloidosis. In contrast, little is known about COOH-terminal fragments of SAA, which

can result from SAA1 cleavage by matrix metalloproteinases (MMPs). Intact SAA1, either on its own or in synergy with chemokines, chemoattracts leukocytes via the GPCR FPR2.

Materials and methods: A novel COOH-terminal fragment of SAA1 [SAA1 (46-112)] was isolated from newborn calf serum. In addition, recombinant intact human SAA1 was cleaved by MMP-9, yielding three COOH-terminal fragments: SAA1 (52-104), SAA1 (57-104) and SAA1 (58-104). We chemically synthesized the COOH-terminal SAA1 fragments SAA1 (46-112), its human equivalent SAA1 (47-104), SAA1 (52-104), SAA1 (58-104) and the complementary NH_2-terminal peptide SAA1 (1-51). The in vitro chemotactic potency on leukocytes of these fragments alone or in combination with chemokines was determined in Boyden microchambers. In vivo recruitment of neutrophils was investigated through intraperitoneal injection of SAA1 fragment in mice.

Results: In contrast to intact SAA1, the COOH-terminal SAA1 fragments and SAA1 (1-51) at 3000 ng/mL failed to directly chemoattract neutrophils and monocytes in vitro. However, the COOH-terminal SAA1 fragments synergized with CCL3 to induce monocyte migration and, comparable to intact SAA1, with CXCL8 to stimulate neutrophil shape change and chemotaxis. In contrast, the NH_2-terminal SAA1 (1-51) lacked this latter potentiating activity. Binding of the COOH-terminal SAA1 fragments to FPR2 was evidenced by a complete blockade of synergy between these fragments and CXCL8 or CCL3 by the FPR2 antagonist WRW_4. Finally, SAA1 (46-112) synergized with CXCR2 ligands to recruit neutrophils to the peritoneal cavity of mice.

Conclusion: Proteolytic cleavage of SAA1 by MMP-9 fine-tunes the inflammatory capacity of this acute phase protein, since only the synergistic interactions with chemokines remain, prolonging the duration of inflammation.

Funding: FWO-Vlaanderen, C1 funding (KU Leuven).

P080-T | Freestyle fluidics—A novel platform to study macrophage chemotaxis

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Background: Chemotaxis is defined as directed cell migration along chemoattractant gradient and is crucial in inflammation and development. Many chemotaxis assays have limited physiological relevance, no flexibility in experimental design and are hard to reproduce. This study aimed to develop a highly reproducible chemotaxis platform that addressed these challenges and to compare it to existing real-time modified Boyden chamber chemotaxis assay.

Material and methods: Freestyle fluidics (FF) is a novel robotic device that prints circuits in any shape using cell media and stabilises them using fluorocarbon oil FC40. Current protocol involves printing three parallel chambers connected to the middle one by 3 channels. Bone marrow-derived macrophages are plated in the middle chamber and after 24 hours rest 10 nmol/L chemoattractant complement C5a is added to one of the other chambers while media is added to the other. A diffusion gradient is established and cells are imaged in real time for 48 hours using IncuCyte Zoom.

Results: Cell migration was quantified with custom-made algorithms, which created histograms of cell distribution throughout the circuit over time and informed on the kinetics of the population and individual cells. FF allowed both population studies and single cell tracking of haptotactic movement over ECM-coated plate, while existing system only showed population migration through non-physiological 8 μm pores. FF circuits were adjusted in size to fit different population sizes and to control the diffusion rate based on the chemoattractant. FF circuits allowed to test multiple conditions simultaneously on the same cell population.

Conclusion: Freestyle Fluidics provides a novel alternative to current real-time chemotaxis systems, allowing multiplicity of designs and real-time imaging of migration under more physiological conditions, all impossible in other systems. We plan to extend our protocols to competition assays between different chemoattractants or cell types by adding additional compartments to the circuits.

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P081-T | A novel BRET-based GTPase assay to investigate the regulation of ARHGAP25

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Background: Small G-proteins regulate numerous immune cell functions. Despite their utmost biological significance, measurement of their GTPase activity with high temporal resolution is a methodological challenge. Therefore we sought to find new ways to reliably assess GTPase activity, possibly in a real-time manner, without the need of radioactive labelling. We hypothesized that active, GTP-bound Rac could be monitored by measuring the distance between Rac and CRIB (Cdc42/Rac interactive binding

domain, which binds active Rac only), using bioluminescence resonance energy transfer (BRET). This method would give us an opportunity to assess the effect of ARHGAP25, which is a key factor in the regulation of phagocytosis, leukocyte migration and extravasation according to our previous results.

Material and methods: Luciferase-tagged CRIB (CRIB-Rluc) and Venus-tagged Rac (Venus-Rac) as well as ARHGAP25 were produced in *E. coli*. BRET was measured every 30 seconds under in vitro conditions in 96-well plates for 15 minutes. (Optimal donor (CRIB-Rluc) to acceptor (Venus-Rac) ratio was determined in advance.) GTPγS or GDPβS-loaded Rac were used to determine BRETmax and BRETmin, resp. Phosphorylation of ARHGAP25 was carried out with TNFα-activated neutrophil cytosol. All results were validated by both radioactive and commercially available endpoint GTPase activity assays.

Results: BRET signal between Venus-Rac and CRIB-Rluc displayed a continuous decrease demonstrating the endogenous GTPase activity of Rac. BRET signal decrease was accelerated by ARHGAP25 in a dose-dependent manner, verifying its effect as a RacGAP. Importantly, phosphorylation of ARHGAP25 with neutrophil cytosol attenuated its GAP activity.

Conclusions: We developed a simple and cost-effective in vitro BRET-based assay for the real-time measurement of Rac GTPase activity. Our method may be tailored to other small GTPases as well. Using this novel approach we demonstrated that phosphorylation of ARHGAP25 reduces its GAP activity.

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P082-T | Tyrosine kinase pathways in monosodium-urate crystal-induced inflammatory responses

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Background: Deposition of monosodium urate (MSU) crystals in the joints or other tissues is a hallmark in the pathogenesis of gout. The urate crystal-induced inflammation is known to be mediated mainly by neutrophils besides monocytes and macrophages, however the MSU crystal-mediated signal transduction is only partially characterized. In this study, we investigated the role of Src family kinases in MSU crystal-induced in vitro activation of primary murine and human neutrophils and their in vivo significance in experimental model of gout.

Material and methods: Bone marrow isolated neutrophils from wild type and triple Src family kinases-deficient (Hck−/−Fgr−/−Lyn−/−) mice or vehicle and Src-inhibitor treated human neutrophils were stimulated with MSU crystals. We examined the superoxide production, cytokine release and phagocytosis of the neutrophils in the presence of the crystals. Gouty arthritis was induced by injection of MSU crystals into the hind paws of the experimental mice and was assessed by ankle thickness measurements and detection of the synovial cytokine levels by ELISA.

Results: The MSU crystal-induced superoxide release, cytokine production and the crystal-phagocytosis were abrogated in Src family kinases-deficient murine or in Src-inhibitor treated human neutrophils. In contrast to wild type animals, Src family kinases-deficient mice showed significantly decreased paw swelling and neutrophil accumulation at the site of inflammation. In line with this, the synovial levels of interleukin-1β and CXCL2 were also strongly reduced in Src family kinases-deficient mice compared to wild type animals.

Conclusions: Src family kinases play an indispensable role in MSU crystal-induced superoxide and cytokine production as well as crystal-phagocytosis of neutrophils and based on our data play role in in vivo gouty arthritis.

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P083-T | Lymphatics modulate the development of contact hypersensitivity

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Contact hypersensitivity (CHS) reaction, the mouse model of human allergic contact dermatitis, can be induced by repeated exposure to contact allergens. The lymphatic system plays an important role for the regulation of the immune response in infectious diseases in the skin but the function of lymphatics in the development of allergic contact dermatitis remains still unclear.

In this study $Flt4^{kd/+}$ mice carrying a germline point mutant kinase dead Vegfr3 allele were used. Our experiments have revealed the complete lack of lymphatics in the skin including the ear of $Flt4^{kd/+}$ mice, while the lymphatic structures were present in the lung and small intestine. CHS was initiated by the exposure of the skin to TNCB (2,4,6-trinitrochlorobenzene)

followed by a second treatment. The disease progression was monitored by ear thickness measurement, H&E histology and immunostaining against lymphatic and immune cell markers. The immune cell infiltration was measured by flow cytometry.

The progress of CHS indicated reduced inflammation in the ear of the $Flt4^{kd/+}$ mice compared to the wild type animal: smaller ear thickness and less immune cell infiltration were detected. Furthermore, the development of CHS induced dynamic changes in lymphatic morphology and resulted in unexpected lymphatic growth with dilated lymphatic structures in $Flt4^{kd/+}$ ears.

Our findings reveal that dynamic changes of lymphatic morphology occur in CHS, and the inflammation is reduced in the $Flt4^{kd/+}$ mice lacking ear lymphatics. Our results also suggest that distinct mechanisms regulate the developmental and inflammatory lymphangiogenic program. Importantly, these results define novel aspects of the interactions between the immune and lymphatic system in allergic diseases.

P084-T | Investigating the role of PLCγ2 in autoimmune skin inflammation

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Background: Epidermolysis bullosa acquisita (EBA) is a human autoimmune skin inflammation caused by autoantibodies against type-VII collagen (C7) which is an essential component of the dermal-epidermal junction. Phospholipase Cγ2 (PLCγ2) is an important member of the immunoreceptor signalling pathways expressed mostly in haemopoietic cells. Our aim was to study the role of PLCγ2 in skin inflammation using the mouse model of EBA.

Materials and methods: Rabbits were immunized by a GST fusion protein of the autoantigenic fragment of C7. IgG from rabbit sera was purified by protein G affinity chromatography. Purity was checked by SDS-PAGE. In the EBA model wild type and PLCγ2 deficient mice were injected subcutaneously with immunized rabbit IgG (anti-C7). Disease progression was followed for 2 weeks by measuring and scoring the affected skin area. Mice were sacrificed and ears were used for histology samples using H&E staining and immunostaining. Neutrophil accumulation in ear samples was measured by flow cytometry. Anti-C7 antibody levels in blood was tested by ELISA.

Results: Wild type mice developed a severe disease after anti-C7 treatment compared to control-treated animals mostly on ears, cheeks and limbs. In contrast, PLCγ2 deficient mice remained unaffected though the amount of circulating anti-C7 antibody was comparable to the wild type. According to histology and flow cytometry a severe dermal immune cell infiltration–mostly neutrophil accumulation–developed in wild type mice. This infiltration was strongly reduced in PLCγ2 deficient animals.

Conclusions: PLCγ2 deficient mice were completely protected from skin inflammation upon anti-C7 treatment compared to wild type animals. Anti-C7 IgG was present in the circulation and showed deposition along the dermal-epidermal junction in both genotypes. However neutrophil accumulation failed to develop in the absence of PLCγ2. These results indicate that PLCγ2 have an essential role in the effector phase of autoimmune skin inflammation.

P085-T | Cross-talk in the tumor microenvironment affects migration and biology of tumor- associated neutrophils

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Background: In patients with malignant disease, neutrophils are recruited into tumor tissue. These tumor-associated neutrophils (TAN) can be localized in mesenchymal, stromal regions of the tumor or in epithelial tumor nests. A high prevalence of neutrophils in the tumor tissue is often associated with poor prognosis in many types of cancer.

Despite this pathobiological importance, it is unclear how neutrophils are recruited to different areas of tumor tissue and whether localization affects the biology of the TAN.

Materials and methods: We used quantitative digital pathology to determine the localization and frequency of TAN in patients with head and neck cancer.

For mechanistic experiments, we established an in vitro system to analyze the cross-talk of neutrophils with tumor cells and tumor-associated fibroblastoid mesenchymal stromal cells (MSC).

Results and conclusions: Our data show a distinct cytokine profile of cancer cells and MSC. This profile is changed upon reciprocal interaction of cancer cells and MSC. This reciprocal interaction also significantly affects migration abilities of neutrophils. Interestingly, exposure of neutrophils to supernatants of MSC, previously exposed to tumor cell-derived factors, led to significant changes in the biology of the neutrophils and prolonged their life span. In patients, TAN were more frequent in stromal regions over epithelial tumor cell nests. Nevertheless, high TAN frequency in tumor core areas, but not in stromal regions, was significantly associated with poor survival.

Our data suggest a cross-talk of tumor cells and stromal cells with functional consequences for the biology of tumor-associated neutrophils.

Funding sources: Marga and Walter Boll Foundation.

P086-T | Characterization of the lymphatic vasculature in atherosclerosis

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Lymphatic vessels are present in the arterial wall, but the physiological and pathophysiological role of these vessels is not fully understood yet. Recently it has been shown that lymphatic vessels participate in the reverse cholesterol transport, suggesting a possible role in the development of atherosclerosis. In this study we aimed to characterize the morphology and growth of the lymphatic vasculature in atherosclerosis.

To visualize lymphatic vessels in the arterial wall, whole aortas of lymphatic reporter mice were cleared by a tissue clearing method, followed by whole mount immunostaining. In parallel, $Ldlr^{-/-}$ and $ApoE^{-/-}$ mice on control or high-fat diet were used to characterize the lymphatic morphology and growth in atherosclerosis. The lymphatic vasculature and plaque formation in atherosclerosis was analyzed by paraffin-based histology followed by H/E-, Oil-Red-O- and immunostaining of lymphatic endothelial markers.

We demonstrated the presence of several lymphatic vessels in the adventitia of the thoracic and lumbar aorta but less lymphatic structures in the aortic arch. Both $Ldlr^{-/-}$ and $ApoE^{-/-}$ mice developed severe atherosclerosis on a high-fat diet, indicating the largest atherosclerotic plaques in the aortic arch. Atherosclerotic mice showed an increased number of lymphatic vessels in the adventitia of the arterial wall in comparison to mice on control diet.

Our results suggest the possible role of the lymphatic vasculature in the development of atherosclerosis. In current experiments we are using genetic loss of function and gain of function approaches to block and stimulate lymphatic growth in atherosclerotic mice. Defining the role of the lymphatic system in the pathogenesis of atherosclerosis may lead to the development of novel therapeutic approaches in the future.

P087-T | Altered composition and proinflammatory function of neutrophil extracellular traps in type 1 diabetes

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Background: Neutrophil extracellular traps (NETs) have been shown to be powerful initiators of inflammation, which has led to the exploration of their role in the pathogenesis of numerous autoimmune diseases, including type 1 diabetes (T1D). Netting neutrophils infiltrate the pancreas prior to T1D onset; however, the precise nature of their contribution to disease pathology remains poorly defined.

Material and methods: To examine how NETs may contribute to the development of T1D, we investigated NET composition and their effects on dendritic cells (DCs), monocytes and T lymphocytes in T1D children.

Results and conclusions: We showed that patient NET composition differs substantially from that of healthy donors; in particular the NET composition of the T1D patients was marked by higher levels of histone-associated DNA and lower levels of antimicrobial proteins. Additionally, the presence of NETs in a mixed peripheral blood mononuclear cell (PBMC) culture caused a strong shift towards IFNγ-producing T lymphocytes in T1D patients but not healthy donors. The NET-induced activation of innate immune cells, demonstrated by the upregulation of costimulatory molecules (CD86) on myeloid and plasmacytoid DCs as well as on monocytes, was observed in both healthy and T1D cultures. Cytokine production was most prominent in healthy control monocytes, whereas samples derived from T1D patients displayed strong cytokine production by both monocytes and dendritic cells. Importantly, in a targeted model of monocyte-derived DC (moDC) culture, NETs once again induced cytokine production, phenotypic change, glycolysis activation and T cell polarization towards IFNγ-producing T cells in samples derived from T1D patients but not in those from healthy donors. In summary, NETs from T1D patients, in contrast to the NETs from healthy donors, have different compositions and promote a distinct proinflammatory response mediated chiefly by dendritic cells.

Funding sources: The study was supported by AZV 16-32838A, the Institutional Support 00064203 and GAUK 460218.

P088-T | Neutrophil function and serine protease activity in the oral cavity

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Background: Neutrophils store several neutrophil serine proteases (NSPs) in granules that form during the maturation process, most notably Elastase, Cathepsin G and Proteinase 3. Activation of NSPs is mediated by Cathepsin C (CTSC) through post-translational truncation. The Papillon-Lefèvre Syndrome (PLS) exhibits a loss-of-function in the CTSC gene leading to diminished CTSC activity, manifesting clinically as a rapid progressing periodontitis. Surprisingly, as NSP activity is usually considered key for microbial killing, this does not lead to a general hypersusceptibility towards bacterial infections. We aimed to characterize PLS neutrophils and elucidate the importance of NSP activity on neutrophil functions.

Material and methods: Neutrophils were sampled from healthy donors, periodontitis patients and PLS patients (n = 4) from peripheral blood and gingival crevicular fluid. Neutrophils and NSPs were characterized with flow cytometry, activity assays, and immunohistochemistry.

Results: Severe periodontitis (n = 3) or peri-implantitis (n = 1) was observed in the participating PLS patients. Both CTSC- and NSP activity was severely reduced and NSPs were largely absent in mature PLS neutrophils. Blood neutrophil counts were unchanged in PLS patients and the neutrophils did not show differences in ROS production, cell death regulation, or degranulation. Sampling of neutrophils from the gingival crevice revealed increased numbers of neutrophils in PLS patients in comparison to periodontitis patients. In vitro, NET formation was altered when stimulating with PMA, but normal when triggered with membrane disturbing peptides.

Conclusions: The importance of active CTSC for activation of NSPs was confirmed. We were able to show that NSP activity is involved in the regulation of certain types of NET formation. PLS neutrophils are described in the literature to have aberrant chemotaxis leading to local tissue neutropenia; gingival samples did not support this assumption.

Funding sources: The Swedish Research Council, the Swedish state (TUA agreement) and The Patent Revenue Fund for Research in Preventive Odontology.

P089-T | Histamine inhibits the capture and killing of bacteria by human neutrophils

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Background: It remains unclear why neutrophil phagocytosis is compromised in Cystic Fibrosis (CF) patients. We hypothesize that histamine produced by bacteria colonizing CF lungs impairs neutrophil phagocytosis. Neutrophils express two types of histamine receptors: the H4R and the H2R. We previously showed that the H4R controls inhibition of neutrophil anti-microbial functions (degranulation). We therefore tested whether histamine impairs neutrophil phagocytosis.

Materials and methods: Neutrophils were isolated from blood of healthy donors, pre-treated or not with a combination of H4R antagonists (10^{-8} to 10^{-6} mol/L) and histamine (10^{-9} or 10^{-6} mol/L) after which leukocytes were exposed to serum-opsonized *E. coli*, *S. aureus*, and *P. aeruginosa* (1:1 ratio). Thereafter, the capture and killing of bacteria were assessed by measuring the concentration of viable bacteria (CFU/ml) in the extracellular medium upon removal of neutrophils or neutrophil lysis.

Galleria Mellonella were injected with *S. aureus* (2.106 CFU/larvae) in the absence or presence of H4R antagonists (10^{-8} to 10^{-6} mol/L) and worm survival was measured over time.

Results: Histamine (10^{-6} mol/L) but no histamine (10^{-9} mol/L) delayed significantly the capture of *E. coli* suggesting that the H2R (low affinity receptor) but not the H4R (high affinity receptor) controls beta 2 integrin- dependent bacteria capture. Histamine (10^{-9} mol/L) significantly delayed the killing of bacteria, an effect mediated by the H4R, since a pre-treatment of these leukocytes with a specific H4R antagonist (10^{-8} to 10^{-6} mol/L) prevented the diamine to block bacteria killing.

Only 30% of Galleria Mellonella survived 48 hours post infection with *S. aureus* and the rate of survival rose to 80% upon injection of the H4R antagonist (10^{-6} mol/L).

Conclusion: Histamine impairs neutrophil phagocytosis. Antagonists of the H4R may be used to improve bacterial clearance in lungs of CF or COPD patients by boosting neutrophil's killing activity.

Funding source: The Medical Research Council (MRC).

P090-T | Receiving the message—Identification of organ-specific 'ZIP-codes' by studying endothelial heterogeneity under different inflammatory stimuli

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The endothelial cell lining shows remarkable heterogeneity. This heterogeneity can be observed on different levels. Furthermore, endothelial phenotype can differ among organs and is dependent on health and disease conditions. The present work aims to compare surface protein on the endothelial lining of different organs in homeostatic and inflammatory conditions. Organ-specific surface molecules will be used to establish 'ZIP-codes' which can be exploited for therapeutic applications. This study concentrates in more detail on the identification of a ZIP-code on the arterial endothelium, which is absent in the microcirculation.

In a bottom-up approach, we isolated, with the use of FACS, endothelial cells from the aorta, bladder, brain, colon, kidney, lung and small intestine from mice in resting conditions and mice experiencing acute inflammation, hypercholesterolemia or diabetes. The obtained ECs are sent for single cell RNA sequencing. Genes that are organ-specific and preferably differ per mouse model will be selected to create ZIP codes. To establish ZIP codes, it will be evaluated if these genes encode for surface molecule expressed on the luminal site of the endothelial cells with the use of CyTOF. Endothelial cells from these seven organs and four mouse models have been sorted and sent for single cell RNA sequencing.

In a top-down approach twelve athero-specific molecules were identified based on a literature search. The expression of six molecules (JAM-1, Cx43, S4, EphB2, NRP-1 and Dll4) was verified in the carotid, bladder and lung of mice in resting conditions and mice experiencing acute inflammation or hypercholesterolemia. Unfortunately, none of these six molecules were specifically expressed.

In summary, we will identify endothelial heterogeneity under different inflammatory stimuli, hereby describing organ-specific ZIP codes, which will provide novel therapeutic targets.

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P091-T | Role of intracellular S100A9 in the regulation of neutrophil pro-inflammatory functions using the myeloid HOXB8 cell line

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Background: S100A8 and S100A9 are Ca2+-binding proteins abundantly expressed in the cytosol of neutrophils. As S100A8/A9 dimers, they are tightly associated to the neutrophil pro-inflammatory functions and are described as damage-associated molecular pattern molecules. When released, S100A8/A9 contribute to the amplification of the inflammation process by recruitment and activation of neutrophils and other inflammatory cell types. At the intracellular level, although it is well established that S100A8/A9 regulate the NADPH oxidase activity, there is no evidence for their implication in other key functions of neutrophils. The aim of this work is to study the role of intracellular S100A9 in the degranulation process and cytokine secretion.

Material and methods: We used in vitro differentiated neutrophils derived from WT and S100A9$^{-/-}$ immortalized murine myeloid progenitors. Characterization of the cellular model and analysis of degranulation process were analyzed by Flow Cytometry. Cytokine mRNAs and secretion were quantified by RT-qPCR and Cytometric Bead Assay, respectively.

Results: We confirmed that ROS production is largely decreased in the absence of S100A9 under inflammatory conditions. In the context of degranulation, a down-regulated expression of CD markers specific for gelatinase granules, secretory vesicles and specific granules was found in S100A9$^{-/-}$ mice. Supplementary experiments are in progress to determine the granule proteins secretion. Moreover, dysregulation of some cytokines (e.g. CCL-4, TNF-a and IL-6) both at the mRNA and secretion levels was observed in absence of S100A9.

Conclusion: These results indicate the potential role of S100A9 in the pro-inflammatory functions of neutrophils. More studies on S100A9-associated mechanisms are required to provide new insights of their involvement in the pathogenesis of inflammatory diseases.

Funding sources: This work was supported by the University of Luxembourg.

P092-T | Neutrophil-specific deletion of the Syk tyrosine kinase abrogates the development of experimental epidermolysis bullosa acquisita

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Background: The inflammatory form of epidermolysis bullosa acquisita (EBA) is triggered by autoantibodies against type VII collagen (CVII), a crucial component of the dermal-epidermal junction. We previously showed that the genetic deficiency of the Syk tyrosine kinase in the hematopoietic compartment protected mice from this partially neutrophil-driven skin inflammation. Here, we investigated if Syk expression was important in neutrophils in the pathogenesis of the experimental form of EBA.

Materials and methods: Neutrophil-specific Syk deletion was achieved by crossing MRP8 promoter-driven Cre recombinase transgenic (MRP8-Cre) animals with Sykflox/flox mice (MRP8-Cre Sykflox/flox, SykΔPMN). Wild type animals were used as controls. The efficacy and specificity of lineage-specific deletion was analyzed by freshly isolated neutrophil or cultured macrophage cell lysates by Western blot. Skin inflammation was triggered by repetitive subcutaneous injection of anti-CVII antibodies. Clinical scoring was based on the specific dermatological abnormalities and the size of the affected skin area. Circulating anti-CVII antibody levels were determined by ELISA.

Results: In contrast to the wild type animals, neutrophils of the SykΔPMN mice did not express Syk, while their macrophages had normal levels of the protein. Compared to wild type animals, mice with neutrophil-specific Syk-deletion were totally protected from skin inflammation triggered by anti-CVII antibodies despite of comparable peripheral anti-CVII levels in the two genotypes.

Conclusion: Our results show an essential role for the Syk tyrosine kinase in the neutrophil compartment in experimental epidermolysis bullosa acquisita.

P093-T | Immunoglobulin A activated myeloid cells induce pathological osteoclast activation in rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is a systemic autoimmune disease that mainly affects the joints. It is characterized by autoantibodies such as anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF). Presence of the immunoglobulin A (IgA) isotype of ACPA and RF has previously been correlated with worse disease prognosis and severe bone erosions in RA patients. Recently, we showed that neutrophils get activated by IgA RF present in serum and synovial fluid of RA patients. Blocking of the IgA Fc receptor (FcαRI) resulted in a significant decrease of cellular functions of neutrophils, suggesting that IgA autoantibodies may contribute to RA pathology. Here we investigate the role of IgA autoantibodies in bone erosion induced by osteoclasts. First we studied the effect of cytokines on osteoclast activity released by myeloid cells upon IgA immune complex (IC) activation. The release of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-8 by neutrophils and monocytes were significantly increased after IgA-IC activation compared to IgG-IC activation. Furthermore, chemoattractant LTB4 was only secreted by neutrophils after IgA-IC activation and not upon IgG-IC activation. Subsequently, osteoclasts were generated in vitro from monocytes stimulated with M-CSF and RANK-L and cultured on bovine bone slices or human collagen plates in the presence of supernatant of IgA-IC or IgG-IC activated neutrophils or monocytes. Resorbing areas were stained with coomassie blue and quantified by Image J. Interestingly, bone resorption was significantly increased when osteoclasts were cultured in the presence of supernatant of IgA-IC activated cells, but not with supernatant of IgG-IC. In addition, we discovered that osteoclasts express FcαRI and may therefore also be directly activated by IgA-IC. Altogether, our results implicates that IgA autoantibody complexes play a role in pathological osteoclast activation inducing joint damage in RA patients. Blocking FcαRI may therefore represent a novel therapeutic strategy for the treatment of RA.

P094-T | Characterization of macrophage populations in the neonatal heart

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Background: Cardiac macrophages (cM) populating the heart during embryo development differ from those in the adult heart[1]. Concurrently, adult and neonatal hearts are very different, with changes in nutrient availability and increase in oxygen concentration that occur immediately after birth leading to metabolic reprogramming of the heart[2]. Here, we have begun the characterization of murine cM from post-natal day 1 (P1) until adulthood to explore if this immune switch

is required for, or driven by the metabolic and mechanical changes of the cardiac tissue.

Material and methods: We have used the macrophage reporter CX3CR1-GFP and mechanical-compromised cMYBP-C3-deficient mice, transmission electron microscopy (TEM) and metabolic analyses to assess the functionality of macrophages in the neonatal heart. To precisely characterize cM populations we performed flow cytometry with a panel of markers (CD64, CD11b, MHCII, Ly6C, CCR2, CD206, CD11c) and t-distributed stochastic neighbor embedding (tSNE) analysis at different post-natal days.

Results: Using flow cytometry we distinguished three macrophage populations: MHCII- CD206lo which are most abundant at P1 and P3, MHCII- CD206hi dominating P5 to P21, and MHCII+, predominant in adulthood. TEM imaging indicates concomitant changes in the shape and size of mitochondria in adult and neonate cardiomyocytes (round vs rod-shaped, respectively), suggestive of metabolic changes. Moreover, in mechanical-compromised adult hearts we found a marked predominance of MHCII- macrophages, suggesting that the mechanical properties of the myocardium may also influence cM phenotype.

Conclusions: Two major changes define cM after birth, one is characterized by the increase in CD206 expression at post-natal stages and the second is the acquisition of MHCII. We will now explore whether the different cM waves are determined by the mechanical properties of the myocardium, and to what extent cM fluctuations guide the metabolic reprogramming of the post-natal heart.

1. Molawi et al., JEM 2014.
2. Gong et al., Science 2015.

P095-T | Unravelling the signalling pathway of CD47-SIRPα checkpoint blockade during neutrophil ADCC

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Background: Recently, we established that neutrophils kill antibody-opsonized tumor cells by a novel cytotoxic process that we termed trogocytosis [Matlung H.L, 2018]. This killing mechanism involves trogocytosis, where fragments of target cell membrane are actively taken up by the neutrophil, thereby disrupting the target cell plasma membrane and eventually killing the cancer cell. Trogocytosis and killing are strictly dependent on antibody-opsonization of the tumor cell, neutrophil Fcγ-receptor signalling and CD11b/CD18 integrin-dependent cytotoxic synapse formation. Furthermore, disruption of CD47-SIRPα interaction further enhances neutrophil cytotoxicity. Although it is known that SIRPα on neutrophils recruits and signals, amongst others, via the tyrosine phosphatase SHP-1, the exact signalling pathway and the proteins that lead to restriction of the neutrophil ADCC remain elusive.

Methods: We used neutrophils from healthy donors or LADIII patients and the pro-myelocytic cell line NB4 as a model mimicking neutrophil function. We used a unique, unbiased screening method for protein-protein interactions, namely Biotin Identification (BioID). BioID utilizes a promiscuous biotin ligase (birA), which biotinylates proteins in close proximity to the protein of interest, that can be further identified by mass spectrometry.

Results: We present evidence that CD47-SIRPα interactions negatively regulate β2 integrin activation in a kindlin-3 restricted way, by using neutrophils from rare LAD-III patients, which lack kindlin-3 expression. To investigate the role of kindlin-3 in neutrophil ADCC, we explored the kindlin-3 interactome using BioID in the NB4 cell line. Identification of the biotinylated proteins followed by STRING analysis revealed proteins that interact with or neighbor kindlin-3, therefore could play a key role in neutrophil ADCC.

Conclusions: Our results disclose an important role of kindlin-3 in neutrophil ADCC during absence of SIRPα-CD47 interaction. By using the above mentioned system, we aim to characterize the pathway(s) downstream of SIRPα and kindlin-3 that control neutrophil-mediated antibody dependent killing of cancer cells.

Funding: KWF.

P096-T | Protective role of a synthetic LipoxinA4 mimetic (sLXm) in an in vivo model of sepsis

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Sepsis is a systemic multi inflammatory condition caused by bacteria overload. This may lead to a lethal outcome either

by the condition itself or its complications. LipoxinA4 is a Formyl Peptide Receptor 2 (ALX/FPR2) binding autacoid, produced during inflammatory states that has shown the ability to promote the natural resolution of inflammation. We have generated a panel of synthetic LipoxinA4 mimetics (sLXMs) and here we have investigated the lead sLXM in a mouse model of sepsis induced by caecal ligation and puncture (CLP) using both wild-type (WT) and FPR2/3$^{-/-}$mice. Following sepsis induction mice were treated twice (1 and 6 hours after surgery) with either the sLXM (2 μg/kg) or vehicle (2% ethanol in saline), and euthanized after 24 hours (n = 6 per group). Heart function was determined by echocardiography, indicating impaired cardiac function in vehicle treated sepsis groups when compared with the sham group. Quantification of ejection fraction, fractional shortening and fractional area change indicated that sLXM treatment initiated partial restoration of normal functionality. Using this model, blood and peritoneal lavage were plated for bacteria count, resulting in markedly diminished number in both samples when challenged with the sLXM. These data suggest a strong indirect antimicrobial activity, potentially mediated via enhanced activation of clearance and killing by the immune system leading to systemic protection. Importantly, there was no evidence of cardiac function restoration or bacteria load decrease in FPR2/3$^{-/-}$mice, indicating that these protective effects are mediated via ALX/FPR2. Finally, analysis of circulating cytokine levels in serum indicated a significant reduction in the levels of several proinflammatory cytokines (IL-6, IFN-γ, TNF-α, IL-10, IL-1β) in WT mice administered sLXm. Taken together, these identify a protective role for sLXMs in sepsis treatment.

P097-T | Differential regulation of biological functions by neutrophilic granulocyte derived extracellular vesicles

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Background: Extracellular vesicles (EVs) transfer biologically active molecules from their cell of origin. Our previous results show that neutrophilic granulocytes (PMN) can release EVs with or without antibacterial properties depending on their activation state. Both pro- and anti-inflammatory effects of PMN derived EVs are reported in the literature. In this study we investigated under comparative conditions the thrombo- and immunomodulatory effects of three different well-characterized PMN derived EV populations.

Materials and methods: Human PMN were stimulated with opsonized particles or left non-activated for 20 minutes. Other PMN were incubated in unstimulated conditions for 24 hours. Cells were eliminated and the medium-sized EV fraction was pelleted via differential centrifugation and filtration. EVs derived from these three different conditions (from activated cells—aEV, spontaneously produced—sEV, from apoptotic cells—apoEV) were co-incubated with leukocytes or pooled human plasma. We evaluated the uptake of the vesicles and their effect on phagocytosis, cell migration, superoxide production, coagulation and cytokine production.
Results: Both sEVs and aEVs were taken up by all investigated cell types. PMN phagocytosis was not affected by the EVs. aEVs seem to slightly enhance the migratory potential of PMN as opposed to sEVs. Superoxide production of PMN was enhanced by aEVs, decreased by sEVs and delayed by apoEVs. apoEVs showed a strong procoagulant effect in recalcified plasma both in the presence and absence of thromboplastin (TP), while sEVs only enhanced coagulation in the absence of TP and aEVs did not have any effect on coagulation. IL-8 secretion of PMN was enhanced by aEVs and decreased by sEVs.
Conclusions: Our data show that human PMN release different EV populations, which have selective and specific—occasionally even opposing—effects on various physiological processes depending on the conditions during their release from the cells.
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P098-T | STING promotes macrophage-mediated resolution of inflammation through IFNβ production

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Background: The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/immune-silencing. Previously, a distinct subtype of resolution-phase macrophages characterized by decreased expression of CD11b, arrest of efferocytosis (satiation) and enhanced reprogramming into pro-resolving and anti-fibrotic phenotypes was identified. These satiated macrophages display increased production and secretion of the immunomodulatory cytokine IFNβ.
Materials and methods: To elucidate the role of the intracellular adaptor protein stimulator of IFN genes (STING) in macrophage production of IFNβ and its consequences, satiated macrophages were sorted from zymosan-A induced

peritonitis and the activation of the STING pathway in these macrophages was examined. In addition, resolution phase macrophages were recovered from WT and STING$^{-/-}$ mice, and analyzed for their cytokine production, efferocytosis, and reprogrammed phenotype using flow cytometry, WB, ELISA and fluorescent microscopy.

Results: Here, we show that satiated macrophages display increased activation of STING, Tank binding kinase (TBK) 1, and IRF3 concomitantly with increased expression of IFNβ and ISG15. However, IFNβ levels were reduced in peritoneal exudates from STING$^{-/-}$ mice. Moreover, activation of the STING-IFNβ pathway, macrophage efferocytosis, reprogramming and responsiveness to apoptotic cells were all diminished in STING$^{-/-}$ resolution phase macrophages, and rescued, at least in part, by exogenous IFNβ.

Conclusions: Thus, our findings indicate that STING is an essential mediator in driving IFNβ expression and secretion by satiated macrophages and consequently in shaping macrophage function and phenotype changes during resolving inflammation.

P099-T | Pro-angiogenic Tie-2 macrophages sustain the peritoneal carcinomatosis shaping inflammatory response in the peritoneal microenvironment

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Background: The peritoneum is a complex immunological microenvironment and the molecular identification of the signals favouring or restricting the ectopic growth of autologous cells is still ongoing. This is particularly true in the case of peritoneal carcinomatosis, a condition characterized by tumour cells growth in the peritoneal cavity occurring because of abdominal tumors. The events that influence the ability of exfoliated cancer cells to attach, to infiltrate the peritoneum, to survive and to recruit vessels are still partially elucidated. Immune cells, like monocytes and neutrophils, are massively recruited in the developing lesions, which depend on novel vessels for establishment and growth. A subset of monocytes/macrophages that express Tie2, the angiopoietin receptor (Tie2 expressing monocytes, TEM) have an established role in angiogenesis.

Material and methods: We have developed a preclinical model of peritoneal carcinomatosis based on the intraperitoneal injection of syngeneic poorly immunogenic adenocarcinoma cells (MC-38) in which selective depletion of TEM was achieved using the Herpes simplex virus type 1 thymidine kinase suicide gene system in C57BL/6 mice.

Results and conclusions: Our preliminary results demonstrate that TEM play a non redundant role in peritoneal carcinomatosis, sustaining the growth and the vascularization of the lesions on one hand and shaping the inflammatory peritoneal environment on the other. The identification at the cellular and the molecular levels of the signals that influence the peritoneal microenvironment and fostering the eventual survival of neoplastic cells derived from abdominal tumors and then the spreading and the growth of lesions might lead to a novel definition of a peritoneal pre-metastatic niche and pave the way to more effective immunotherapeutic strategies.

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P101-T | Involvement of voltage-gated potassium channels in pinacidil effects on the isolated bypass grafts from patients with type-2 diabetes mellitus

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In recent years there has been an increasing trend in the number of coronary artery bypass grafts (CABG) surgeries in the patients with type-2 diabetes mellitus (T2DM). In diabetic patients have been observed reduction in relaxation of blood vessels which could be result of different expression and/or function of smooth muscle potassium (K) channels. Pinacidil, a potassium channel opener (PCO), induced potent endothelium-independent relaxation of bypass grafts and its mechanism of relaxation is partly correlated with an interaction with smooth muscle ATP-sensitive potassium channels, but also included other types of K channels. Thus, the objective of our study was to investigate the involvement of voltage-gated K (Kv) channels in the effect of pinacidil on human saphenous veins (HSV) and human internal mammary arteries (HIMA) obtained from patients with T2DM.

Rings of HSV and HIMA, without endothelium, were mounted in organ bath system and isometric tension was being recorded. Pinacidil (0.01-100 μmol/L) was used for relaxation of HSV and HIMA precontracted with phenylephrine (0.1 mmol/L) and 5-hydroxytryptamine (0.1 mmol/L), respectively.

Pinacidil produces concentration-dependent vasorelaxation of HSV and HIMA obtained from diabetic patients. 4-aminopyridine (4-AP, 1 and 3 mmol/L), a nonselective blocker of Kv channels antagonize the effect of pinacidil on HIMA obtained from patients with T2DM ($P < 0.05$ both). The same concentrations of 4-AP did not antagonize the effect of pinacidil on HSV obtained from patients with T2DM ($P > 0.05$ both). Margatoxin, highly selective blocker of Kv1.3 channels (30 nmol/L), did not change relaxation effects of pinacidil on both types of grafts obtained from patients with T2DM ($P > 0.05$).

It seems that 4-aminopyridine-sensitive Kv channels are involved in the vasodilatation of HIMA induced by pinacidil. The Kv1.3 channels do not contribute to the relaxation effects of pinacidil on bypass grafts obtained from the patients with T2DM.

P102-T | Effect of dopamine on action potential generation in the newborns rats right atrium cardiomyocytes

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Background: Dopamine is known as the major neurotransmitter in CNS. Dopamine was also found in the sympathetic ganglia, nerves and heart. The intensity of dopamine secretion is 10-20 times higher than that of epinephrine and norepinephrine. The effect of dopamine on the heart in low concentration is mediated via dopamine receptors and in high concentration via α- and β-adrenoceptors. The purpose of this study is to investigate dose-dependent dopamine effects on the parameters of right atrial preparations electrical activity in newborns rats.

Methods: The study was carried out on 7-day white outbred laboratory rats. Membrane potential (MP) and action potential (AP) were recorded using glass microelectrodes (tip diameter <1 μ, resistance 30-80 MΩ).

Results: Dopamine caused concentration-dependent changes in the electrical activity. Dopamine at a concentration of 10^{-7} mol/L did not cause significant changes in MP and PD parameters of animals. Dopamine at a concentration of 10^{-6} mol/L did not cause changes in MP, AP amplitude and the duration of depolarization. But the duration of repolarization increased by 12% ($P < 0.05$). Dopamine at a concentration of 10^{-5} mol/L did not cause significant changes in MP, AP amplitude and the duration of depolarization. The duration of the repolarization increased by 22% ($P < 0.05$).

Conclusions: Our studies indicate that dopamine receptors are involved in regulating of the AP repolarization phase duration. The increase of the repolarization phase duration is possibly associated with a change in the kinetics of K+ channels and total K-current decrease. Further studies will determine the subtypes of dopamine receptors involved in the regulation of the cardiomyocytes electrical activity.

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P103-T | The influence of clonidine hydrochloride on the myocardium electrical activity

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The presence and functional role of α2-AR in the humans and animals heart was extensively studied. This work assessed changes of the action potential (AP) parameters of rat atrial cardiomyocytes induced by a α2-adrenoreceptor agonist clonidine hydrochloride in concentrations 10^{-9} to 10^{-5} mol/L.

The experiments on intracellular recording of electrical activities in the working myocardium, were carried out on random-bred albino rats. Isolated right atrial wall from the right auricle exhibiting no pacemaker activity was placed in a 3-mL chamber and superfused with Tyrode solution at 38°C at a rate of 10 mL/min. Intracellular AP was recorded via glass microelectrodes with resistance of 40-80 MΩ. The signals were digitized with an E14-140 converter (L-Card). The data were processed with Elph 3.0, Microsoft Excel software and Student's *t* test.

Clonidine hydrochloride in concentrations of 10^{-9} to 10^{-5} mol/L increased the duration of the action potential and reduced the frequency of action potential generation. The maximum concentration of the substance caused the maximum effect. None of the tested concentrations of the clonidine hydrochloride produced significant effect on resting potential or upstroke velocity of action potential.

Stimulation of α2-AR clonidine hydrochloride affects the electrical activity of the right atrial cardiomyocytes in adult rats and the duration of the action potential, repolarization phase continuance and the frequency of action potential generation.

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P104-T | Comparative analysis of the influence of If blockade on newborn and adult rats Langendorf-isolated heart

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HCN channels regulate cardiac rate control altering the activity of hyperpolarization activated currents (If). This study performed comparative analysis of the influence of If blockade on the Langendorf-isolated heart of newborn and adult rats.

Ex vivo experiments were done on white outbred newborn rats without sympathetic innervation of the heart. Adult animals with a formed system of autonomous regulation of the heart were chosen as a control group. Isolated heart was perfused in the installation of Langendorff (ADinstruments) Krebs-Henseleit solution. The coronary flow (CF) and heart rate (HR) were calculated along the curve. The signals were recorded in a PowerLab system (ADinstruments) with the help of LabChart Pro 8.0 software. ZD7288 (Sigma) at the concentrations range of 10^{-9} to 10^{-5} mol/L has been used for If current blockade. The data analyzed using Student's *t*-test. ZD7288 10^{-9} mol/L decreased of newborn rats HR by 27% ($P \leq 0.05$). ZD7288 10^{-8} to 10^{-5} mol/L caused multidirectional effects of newborn rats HR. The If blocker 10^{-5} mol/L reduced CF of the newborn rats isolated heart by 10% ($P \leq 0.01$). Other concentrations of the blocker did not influence of newborn rats CF. In the control group ZD7288 10^{-9} mol/L and 10^{-6} mol/L decreased HR by 25% ($P \leq 0.01$), and 22% ($P \leq 0.01$), respectively. If blocker 10^{-9} mol/L reduced CF of adult animals by 21% ($P \leq 0.001$). ZD7288 in concentrations of 10^{-8} to 10^{-5} mol/L did not change CF in adult animals.

If blockade affected newborn and adult rats isolated hearts HR and CF. The dose-dependent effect of ZD7288 was different in newborn and adult animals.

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P105-T | The α2B-adrenoceptor selective blockade influence on newborn rat myocardium inotropy

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Previous studies showed α2-adrenergic receptors role in various physiological functions in the regulation of the cardiovascular system and central nervous system. All three subtypes of α2-adrenergic receptors have been found in rats cardiac tissue including right atrium and left ventricle. The maximum expression of α2-adrenergic receptors in rats heart noted in fetal cardiac tissue. The goal of the study was to perform comparative analysis of α2B-adrenergic receptors blockade influence with imyloxan hydrochloride on the inotropy of atrium and ventricle myocardium in newborn and adult rats.

The rats were anesthetized with intraperitoneal injection of urethane. Registration of isometric contraction of ventricular and atrial myocardial strips rats was carried out on the MP-150 installation (BIOPAC Systems). To block α2B—adrenergic receptors, imyloxan hydrochloride was used in the concentrations of 10^{-5} to 10^{-9} mol/L.

All studied concentrations of imyloxan hydrochloride induced negative effect on newborn rats atria and ventricular contraction force. The blockade of α2B-adrenoreceptors in all concentrations resulted in positive inotropic effect in the adult rats atria and in the ventricles. The change of contraction force after α2B-adrenoreceptors blockade in the ventricles was more pronounced than in the atria.

Thus, the effect of α2B-adrenoreceptors blockade with imyloxan hydrochloride depends on the animal age.

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P106-T | The influence of α1A-ARs inhibition on the isolated heart chronotropy and coronary flow

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Alpha1-adrenergic receptors (α1-ARs) participate in many adaptive processes. The important effect of stimulation of α1-ARs is constriction of blood vessels and increase in arterial blood pressure. α1A and α1B subtypes of adrenoreceptors are densely represented in the myocardium, while α1D-ARs are found in smooth muscle cells and coronary arteries. The role of α1-ARs in the regulation of the heart rhythm is confirmed by previous studies, which showed that stimulation of α1-ARs with methoxamine decreases the HR of the 20-week-old rats hearts. The goal of this study was to assess the effect of blockade of α1A-ARs on isolated heart of 1- and 20-week-old rats.

The rats were anesthetized intraperitoneally with 25% urethane (800 mg/kg body weight). The heart was perfused in the Langendorf system (ADInstruments) with a Krebs-Henseleit solution at 37°C and constant hydrostatic pressure of

60-65 mm Hg. To blockade of α1A-ARs, WB4101 (Sigma) was used at a concentration of 10^{-6} mol/L. The coronary flow (CF) and heart rate (HR) were calculated along the curve. Statistical analysis was carried out with Student's *t*-test.
The selective antagonist of α1A-ARs, WB4101 10^{-6} mol/L decreased HR by 19% ($P < 0.01$) and induced an increase of CF by 15% in 20-week-old rat hearts. Selective blockade of α1A-adrenoreceptors had no effect on the isolated heart of 1-week-old rats.
The results indicated that the functional activity of the α1A-ARs in the rats heart has significant age-related features.
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P107-T | Effect of sodium nitroprusside on the rats myocardial contractility after NO-synthase blockade

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Background: NO-synthases present in various cardiac structures playing the important role in conduction of cardiomyocytes and contractility of coronary vasculature. NO production is regulated by NO-donors and several inhibitors of NO-synthases, which can selectively affect certain cells and tissues. Sodium nitroprusside (SNP) is a commonly use NO donor.
Material and methods: Experiments were carried out on random-bred albino rats. The contractile activity of myocardium was examined in vitro in a PowerLab setup equipped with a MLT 050/D Force Transducer (ADInstruments). The contractility of the myocardium strips of the rats atria and ventricles in the presence of the NO donor—sodium nitroprusside (SNP at a dose of 10^{-6} mol/L) and NOS inhibitor L-NAME at a dose of 10 mg/kg was studied. We evaluated the contractility of the atria and ventricular myocardium of rats to NO donor sodium nitroprusside (SNP) at a dose of 10^{-6} mol/L, and effect of SNP after administration of NOS inhibitor—L-NAME (10 mg/kg).
Results: In control group SNP increased the contractile force (CF) of ventricular myocardial strips by 24.64 ± 2.1%. In control group SNP decreased the CF of atrial strips by 12 ± 1.3%. In experimental group after inhibiting of NOS with L-NAME, SNP increased CF of ventricular myocardial strips and atrial stripes by 32.5% and 4% respectively.
Conclusions: SNP increased rat ventricular myocardial contractility and decreased the CF of atrial strips. L-NAME affected atrial contractile response to SNP.
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P108-T | Effects of ATP on action potentials in the atrial and ventricular myocardium of the rat heart during early postnatal ontogenesis

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Extracellular purines play major roles during embryogenesis, organogenesis, and postnatal development. Also, ATP is a sympathetic is a cotransmitter. P2X-receptors on cardiomyocytes sarcolemma are cation-selective ionic channels characterized by virtually equal permeability for Na+ and K+ ions and pronounced permeability for Ca2+ ions. These channels open in response to micromolar concentration of extracellular ATP and are responsible for rapid cell response to ATP. The research aim is to investigate dose-dependent effects of the ATP on the parameters of electrical activity right atrial and ventricular preparations of 7-, 21-, 100 day-old rats.
Methods: The study was carried out on 7-, 21-and 100-day rats. Membrane potential (MP) and action potential (AP) were recorded using glass microelectrodes. The stimulus duration (1 ms) and repetition rate (5 Hz). Statistical significance was assessed using Student's *t* test.
Results: ATP at a concentration of 10^{-9} mol/L did not cause significant changes in MP and AP parameters in all age groups of animals. ATP (10^{-8} to 10^{-5} mol/L) caused a concentration-dependent change in the electrical activity of the rats right atrium and ventricular myocardium. ATP (10^{-8} to 10^{-5} mol/L) quickly reduced AP duration at 20, 50, 90% of repolarization (APD20, 50, 90) at 7-, 21, 100-days rats. The maximum effect APD20, 50, 90 on extracellular ATP was observed in 7-day rats (10^{-7} mol/L), and in 21 day rats (10^{-6} mol/L) ($P < 0.05$).
Conclusions: Our results indicate that purinoreceptors reduced the AP duration of the repolarization. The threshold concentration of the ATP from 7 to 100 days old rises, indicating a decrease in the density and sensitivity of purinoreceptors of cardiomyocytes to agonist. The effects of ATP are most pronounced in rats 7 and 21-days age, which are

characterized by the immaturity of sympathetic regulatory effects on the heart.

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P109-T | NPY regulates electrical activity atrial and ventricle cardiomyocytes in postnatal ontogenesis of rats

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Background: Neuropeptide Y (NPY) is released from sympathetic neurons and exerts short-term effects on prejunctional nerve terminals and postjunctional cardiac ion channels. NPY also exerts trophic effects on angiogenesis, cardiac hypertrophy, autonomic signaling, cardiac ion channels, including effects on L-type Ca2+ and pacemaker channels. Results suggest a long-term influence of NPY to modify the autonomic sensitivity of the heart and/or the ionic channels that are the target of NPY agonists. The research aim is to investigate dose-dependent effects of the non-selective NPY on the parameters of electrical activity of rat right atrial and ventricular preparations.

Methods: The study was carried out on 7-, 21-and 100-day rats. Membrane potential (MP) and action potential (AP) were recorded using glass microelectrodes. The stimulus duration (1 ms) and repetition rate (5 Hz). Statistical significance was assessed using Student's *t* test.

Results: NPY at a concentration of 10^{-9} mol/L did not cause significant changes in MP and AP parameters in all age groups of animals. NPY reduced AP duration at 20, 50, 90% of repolarization (APD20, 50, 90) at 7-days rats at a concentration of 10^{-8} and 10^{-7} mol/L. NPY reduced APD 50, 90 at 21-days rats at a concentration—10^{-8} and 10^{-6} mol/L and in 100-days rats—10^{-6} mol/L ($P < 0.05$).

Conclusions: Our results indicate that NPY-receptors changes the AP duration of the repolarization. The threshold concentration of the peptide from 7 to 100 days old rises, indicating a decrease in the density and sensitivity of NPY receptors of right atrial and ventricular cardiomyocytes to agonist. The effects of NPY are most pronounced in rats 7 and 21-days age, which are characterized by the immaturity of sympathetic regulatory effects on the heart.

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P110-T | The effect of blockade VIP-receptors on myocardial contractility in rats

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Background: The 28-amino acids vasoactive intestinal peptide (VIP) was initially isolated from the intestine and identified soon thereafter as a neuropeptide localized both in the central and peripheral nervous system. VIP belongs to a family of structurally related neuropeptides and hormones that include secretin, glucagon and the closely related with pituitary adenylate cyclase-activating polypeptide PACAP. VIP is expressed by neurons in various brain areas, - and stored and released from nerve fibers innervating numerous organs, including heart, lung, thyroid, kidney, urogenital and gastrointestinal tracts. There are differences in the localization of the three VIP/PACAP receptors. VPAC1 is expressed in brain and in peripheral tissues such as liver, lung and intestine. VPAC2 is expressed in the CNS and in a number of peripheral tissues, including the heart, blood vessels, skeletal muscle and others. PAC1 is present predominantly in brain, in the adrenal medulla. The wide distribution of these receptors indicates that VIP/PACAP affect many different targets, both in the CNS and in the periphery. The research aim is to investigate dose-dependent effects of the blockade of VIP/PACAP receptors in the heart contraction.

Methods: Registration of isometric contraction of right atrial preparations with their own rhythm was carried out on a PowerLab device with a force sensor MLT 050/D (ADInstruments).

Results: The non-selective antagonist of VIP-receptors (10^{-10} mol/L) [Ac-Tyr1, D-Phe2]-VipAntagonist-GRF produced decrease in own rhythm frequency and myocardial contractility ($P < 0.05$). VipAntagonist 10^{-9} mol/L, 10^{-8} mol/L produced a biphasic effect: in first the increase ($P < 0.05$) and then the decrease in own rhythm frequency and myocardial contractility ($P < 0.05$). VipAntagonist-GRF 10^{-7} mol/L did not significantly affect the studied parameters.

Conclusions: Our results indicate that the blockade of VIP-receptors causes significant changes of own rhythm frequency and myocardium contractility.

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P111-T | Clinical usefulness of abdominal bioimpedance (ViScan) in the determination of visceral fat in lean, overweight and obese subjects

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Background: Visceral adipose tissue (VAT) has been shown to be strongly associated with the development of obesity-associated comorbidities. The measurement of VAT usually implies the use of costly and time consuming imaging techniques such as computed tomography (CT) or magnetic resonance imaging. The aim of the present study was to evaluate the accuracy of the determination of VAT using abdominal bioimpedance (BIA) with the ViScan device (TANITA AB 140) in comparison with CT.

Material and methods: We studied a cohort of 140 individuals (73 men/67 women) with BMI ranging from 17.7 to 50.4 kg/m^2 to evaluate the accuracy of the ViScan in comparison to CT to determine VAT. To further analyse ViScan's clinical usefulness we studied a second cohort (n = 2849) studying cardiometabolic risk factors. Furthermore, we analysed the ability of the ViScan to measure changes in VAT after weight gain (n = 107) or weight loss (n = 335).

Results: ViScan determines VAT with a good accuracy in individuals with a CT-VAT lower than 200 cm^2, and then with lower precision with increasing body mass, exhibiting a moderate-high correlation with CT-VAT ($r = 0.75$, $P < 0.001$). Interestingly, VAT determination with the ViScan exhibits better correlations with several cardiometabolic risk factors such as glucose, triglycerides, HDL-cholesterol and markers of liver steatosis than anthropometric measurements such as BMI or waist circumference. ViScan is able to detect VAT variations after body weight changes.

Conclusions: Since the possibility of measuring VAT by imaging techniques is not always available, abdominal BIA represents a good alternative to estimate VAT, allowing the identification of patients with increased VAT-related cardiometabolic risk.

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P112-T | Effective hepatoprotective agent based on sodium-, calcium-, ironpolygalacturonate

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Background: The development of effective nontoxic drugs for the liver treatment and recovery is actual problem. The plant-derived polysaccharides including pectins acquire greater importance for it solution. Previously water-soluble metal complexes based on citrus pectin with biogenic macro- and micromolecules (Fe and Ca) was synthesized and characterized by modern physical chemical methods (NMR, IR, DLS, AFM). In previous studies the toxicological safety of sodium-, calcium-, ironpolygalacturonate (PG Na, Ca, Fe) was shown (LD50 value was estimated greater than 5000 mg/kg per os in rabbits) [1].

Material and methods: The present study reports in vivo performance evaluation the action of PG Na, Ca, Fe in the dose of 60 mg/kg during 10 days after administration of hepatotropic carbon tetrachloride that caused the liver and blood cells damage.

Results: PG Na, Ca, Fe administration to male rats leads to full or part normalization studied parameters: body temperature in terminal stage of toxic damage is raised, liver mass coefficient decreased, the square of damaged liver tissue decreased, the level of ferment activity (specified markers of liver damage) decreased (aspartate aminotransferase—in 4.4 times and lactate dehydrogenase—in 2 times), the level of alanine aminotransferase decreased as well. The results were statistically significant at $P < 0.05$. PG Na, Ca, Fe normalizes metabolic indicators after exposed toxic carbon tetrachloride. Protein, glucose and cholesterol are increasing to the level of physiological norm.

Conclusions: The hepatoprotective properties of PG Na, Ca, Fe consisting in decrease of functional and structural liver damages at toxic hepatite were demonstrated.

[1] Minzanova S.T., et al. Toxicology reports. 2018. 5:457-467.

P116-T | Elevated parathyroid hormone is associated with an increased mortality risk in type 2 diabetes

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Background: Parathyroid hormone (PTH) is one of the main regulators of calcium homeostasis. PTH levels are elevated in primary hyperparathyroidism as well as with vitamin D deficiency or chronic kidney disease. The association of increased PTH levels with all-cause mortality in high-risk patients is unclear.

Material and methods: We therefore investigated the impact of serum PTH on mortality risk in a large series of 939 patients undergoing coronary angiography for the evaluation of established or suspected coronary artery disease (CAD), including 244 patients with type 2 diabetes (T2DM). Prospectively, deaths were recorded over a mean follow-up period of 6.2 years.

Results: PTH at baseline was inversely associated with eGFR (rho = −0.228; $P < 0.001$) and 25-hydroxy-vitamin D (rho = −0.243; $P < 0.001$) and was positively associated with age (rho = 0.122; $P < 0.001$) and BMI (rho = 0.099, $P = 0.002$). Prospectively, elevated PTH was not significantly associated with an increased mortality risk in the total study cohort (standardized HR 1.30 [0.96-1.76]; $P = 0.092$). However, subgroup analysis with respect to T2DM showed a highly significant association of PTH with mortality in patients with T2DM (HR 2.32 [1.37-3.95]; $P = 0.002$), but no association of PTH with mortality in non-diabetic subjects (HR 1.04 [0.82-1.32]; $P = 0.766$). An interaction term T2DM x PTH was significant ($P = 0.006$), indicating a significantly stronger influence of PTH on mortality risk in patients with diabetes than in individuals without T2DM. The impact of PTH on mortality risk in patients with T2DM remained significant after adjustment for age, gender, and BMI (HR 2.30 [1.34-3.93]; $P = 0.002$) as well as after additional adjustment for smoking, kidney function, baseline vitamin D and angiographically determined baseline CAD (HR 1.91 [1.07-3.40]; $P = 0.029$).

Conclusion: We conclude that elevated PTH levels are a strong and independent predictor of all-cause mortality in patients with T2DM.

P117-T | Role of leptin in hepatic inflammation and extracellular remodelling via nitric oxide

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Introduction: The hepatic extracellular matrix (ECM) remodelling during fibrosis in non-alcoholic fatty liver disease (NAFLD) involves the synthesis, secretion and degradation of different matrix components, including tenascin C (TNC). The aim of the present study was to analyze the influence of inducible nitric oxide synthase (iNOS) gene deletion on inflammation and ECM remodelling in the liver of ob/ob mice given that a functional relationship between leptin and iNOS has been defined.

Methods: The expression of molecules involved in liver inflammation and ECM remodelling were analyzed in double knockout (DBKO) mice simultaneously lacking the ob and the iNOS genes. Moreover, the effect of leptin replacement was analyzed in control, leptin-treated (1 mg/kg/d) and pair-fed ob/ob mice, and compared to wild types (n = 50).

Results: The absence of the ob gene increased ($P < 0.01$) liver inflammation and fibrosis. As expected, leptin administration corrected the obese phenotype of ob/ob mice, whereas the simultaneous absence of both iNOS and leptin improved insulin sensitivity, liver inflammation and fibrogenesis, as evidenced by lower macrophage infiltration and collagen deposition, as well as the downregulation of important proinflammatory and profibrogenic genes including Tnf ($P < 0.05$), Emr1 ($P < 0.01$), Hif1a ($P < 0.01$), Col1a1 ($P < 0.01$), Col6a1 ($P < 0.01$), Col6a3 ($P < 0.01$), Spp1 ($P < 0.01$), Cd44 ($P < 0.01$) and Tnc ($P < 0.05$). Circulating TNC levels were also decreased ($P < 0.05$). Moreover, leptin upregulated ($P < 0.05$) TNC expression and release via NO-dependent mechanisms in AML12 hepatic cells.

Conclusion: Ablation of iNOS improved hepatic inflammation and ECM remodelling-related genes of ob/ob mice by decreasing fibrosis and metabolic dysfunction. The synthesis

and release of profibrogenic and proinflammatory TNC depend on iNOS activation induced by leptin, suggesting an important role of this alarmin in the development of hepatic inflammation and fibrosis in the obese state.

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P118-T | Vascular ATP-sensitive potassium channels as a site of action for P1075 in patients with and without type 2 diabetes mellitus

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Background: It is known that ATP-sensitive potassium channels (K-ATP) play important role in regulation of vascular tone and glucose homeostasis. Under psychological conditions, relaxation of blood vessels is highly correlated with the intact endothelium and the activity of K-ATP in vascular smooth muscle. However, long-term presence of type 2 diabetes mellitus (T2DM) causes significant endothelial injury and attenuation in blood vessel response to different vasoactive compounds. As disease progresses, morphological changes of blood vessels and decreased activity of vascular smooth muscle K-ATP lead to functional changes and many of patients with T2DM eventually become candidates for coronary artery bypass grafts (CABG) surgery. The objective of our study was to investigate difference between relaxation of human saphenous vein (HSV) obtained from patients with and without T2DM, induced by P1075, a selective K-ATP channels opener.

Material and methods: Samples of HSV (without endothelium) were taken after coronary arteries bypass grafting (CABG) of patients with and without T2DM. After mounting in system for isolated organs isometric tension was recorded. P1075 (1-100 μmol/L) was used for relaxation of HSV precontracted with phenylephrine (0.1 mmol/L).

Results: P1075 had similar relaxant effect in groups of patients without (EC50 = 1.1 μmol/L) and with (EC50 = 1.4 μmol/L) T2DM, showing no statistically significant difference. A highly selective K-ATP channels blocker, glibenclamide (10 μmol/L) potently antagonized the effect of P1075 in HSV from patients without (EC50 = 0.24 mmol/L) or with (EC50 = 0.28 mmol/L) T2DM ($P < 0.05$, both).

Conclusions: In a both experimental models P1075 caused comparable relaxation of HSV obtained from patients without/with T2DM. It seems that glibenclamide-sensitive K-ATP channels are involved in the vasodilatation of HSV induced by P1075.

P119-T | Crosstalk between inflammation and hemostasis in acute pancreatitis patients

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Background: Mortality in severe acute pancreatitis (SAP) remains high, despite advances in intensive care and surgical treatment. Processes of inflammation and coagulation are enhanced each other and played the important role in the pathogenesis of severe forms of pancreatitis.

Materials and methods: The work is a comprehensive study, which assessed the features of the inflammatory process and hemostasis in 98 patients with moderately severe acute pancreatitis (MSAP) and 57—SAP.

Results: Changes in hemostasis in patients with MSAP and SAP could be described as acquired thrombophilia, characterized by primary activation of hemocoagulation factors, deficiency of natural anticoagulants and fibrinolytics, and activation of intercellular interaction. It was determined that hypercoagulable factors in the blood of patients with AP enhanced the effect of each other. Proinflammatory factors also stimulated, as a rule, the synthesis of each other and suppressed the production of antiinflammatory. Using discriminant analysis, it was found that in the development of renal failure the leading role is played by a decrease in AT III activity and high concentrations of IL-6 and TNF-α, pulmonary dysfunction—a high soluble fibrin-monomer complexes (SFMC) and lengthening of thrombin time (TT), cardiovascular failure—an increase percentage of activated platelets, inhibition of fibrinolysis, lengthening of TT. According to the ROC curve, the sensitivity of determination of SFMC for prediction of pulmonary dysfunction in patients with AP was 86.2%, and the specificity was 83.8%, with positive and negative predictive values of 80.65% and 88.57%, respectively (cut-off level 137.50 mg/L). The sensitivity of determining IL-6 for predicting renal dysfunction was 76%, and specificity—78% (cut-off level 111.30 pg/mL)

with positive and negative predictive values of 67.86% and 84.21%, respectively.

Conclusion: The mechanism of the organ dysfunction following acute necrotizing pancreatitis is complicated. The inflammatory cascades and hypercoagulative state are initiated this pathological process.

P120-T | Increased levels of IL-32a in obesity are associated to colon cancer development by promoting adipose tissue inflammation

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Introduction: Adipose tissue inflammation is an important mechanism whereby obesity promotes colon cancer (CC) risk and progression. Interleukin (IL)-32 has been proposed as an important inflammatory and remodelling factor in obesity and has been also related to CC development. Our aim was to analyze whether obesity can influence the circulating and expression levels of IL-32α in patients with CC, thereby promoting a microenvironment favourable for tumor growth. Moreover, we evaluated the role of IL-32 in inflammation in human CC cells.

Methods: Visceral adipose tissue (VAT) gene and protein expression levels as well as circulating concentrations of IL-32α were analysed in 84 subjects (27 lean and 57 obese) further subclassified in 49 without CC and 35 with CC. The effect of IL-32α on the expression levels of inflammation and ECM remodelling related-genes in HT29 cells was also explored.

Results: We show that obesity ($P = 0.009$) and CC ($P = 0.026$) increase circulating concentrations of IL-32α. Gene ($P < 0.05$) and protein ($P < 0.01$) expression levels of IL-32α were upregulated in obese patients with CC compared with obese patients without CC. Inflammation-related factors and hypoxia significantly enhanced ($P < 0.01$) the expression of IL32A expression in human HT29 cells. The treatment of CC cells with IL-32α significantly enhanced the mRNA levels of the inflammatory factors TNF ($P < 0.01$), CCL2 ($P < 0.05$) and IL8 ($P < 0.05$). We also found a strong upregulation ($P < 0.001$) of the ECM remodelling genes SPP1 and MMP9 after IL-32α treatment in HT29 cells. A significant increase ($P < 0.05$) in CC cells stimulated with the adipocyte-conditioned medium obtained from obese volunteers was observed.

Conclusion: The upregulated levels of IL-32 in patients with obesity and CC as well as its capacity to upregulate pro-inflammatory and ECM remodelling genes suggest the involvement of IL-32 in the development of obesity-associated CC.

No conflict of interest

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P121-T | The contractile activity of resected colon preparations of patients with chronic constipation

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Background: Chronic constipation (CC) is a prevalent, heterogeneous pathologies of gastrointestinal system, the frequency of which increases with age. Etiology is of CC remains poorly understood and is most likely multifactorial and may include a sedentary lifestyle, the use of harmful products, unfavorable environmental conditions and failure of gut motor function. As a consequence the accumulation of feces, severe pain and endotoxicosis caused by increased permeability of the intestinal barrier are observed. The aim of this study was to characterize spontaneous and carbachol-evoked contractions of colonic strips from CC patients.

Materials and methods: The colonic tissue samples were obtained from patients undergoing colectomy for CC and patients undergoing colorectal surgery for gut diseases not associated with disorders of motor function (control group). Contractile activity of the colon stripes was analyzed under isometric conditions using Biopac (USA).

Results: The colon segments had spontaneous activity of varying intensity in both group. In some preparations, spontaneous contractile activity was not recorded. KCl (70 mmol/L) and carbachol, agonist of muscarinic cholinergic receptors at concentrations of 0.01, 0.1, 1, 10, 100, 200 μmol/L, were used to induce the contraction of the colonic segments. The cumulative addition of carbachol at concentrations of 1-200 μmol/L led to a dose-dependent increase in the amplitude of contractions. The comparison of dose-dependent curves in control and CC groups

revealed the higher sensitivity of muscles from CC group to carbachol.

Conclusion: Our results suggested that both the longitudinal and circle muscles of the colon from CC patients were more sensitive in carbachol and KCl indicating on the preserved motor functions of smooth muscle cells. Hypersensitivity to cholinergic stimulation apparently may be explained by the development of a secondary denervation syndrome, which is consistent with anomalies of intramural plexuses.

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P122-T | The effects of short-chain fatty acids on spontaneous colon motility of mice with irritable bowel syndrome

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Background: Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders defined as a variable combination of chronic or recurrent gastrointestinal symptoms without any structural or biochemical abnormalities. Recently, it was suggested that alterations in the gut microbiota, leading to abnormal intestinal fermentation of short-chain fatty acids (SCFAs) may impact in IBS pathogenesis through effects on intestinal secretion and motility. However, the mechanisms of SCFAs action on the colonic motility are not fully elucidated. In this study, we investigated SCFAs effects on mouse colon motility in control and IBS.

Materials and methods: Experiments were performed on mice randomly assigned to either a control or a model group. IBS was induced by intracolonic infusion of 1% acetic acid in saline. The motility of isolated colon segments was studies under isometric conditions in the organ bath setup (Biopac, USA).

Results: Sodium acetate, sodium propionate and butyrate were added cumulatively to the bath in concentrations 0.5, 1, 5, 10 and 30 mmol/L. 0.5 and 1 mmol/L SCFAs did not induce significant changes in the parameters of contractile activity. At 10 mmol/L SCFAs demonstrated inhibitory effects and at 30 mmol/L induced a complete blockade on spontaneous contractile activity in the control group. However, in the IBS group the colon segments were less sensitive to inhibitory action of SCFAs.

Conclusion: Thus, we have shown that SCFAs have different effects on colon motility in normal and pathological conditions. It was suggested that in normal conditions SCFAs can modulate the contractile activity of the intestine by regulating tonus, frequency and amplitude of contractions. The impaired sensitivity of colon to SCFAs in IBS may play role in disease pathogenesis.

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P123-T | Cholecalciferol supplementation for regulation of cholesterol level in PCOS

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Background: Polycystic ovary syndrome (PCOS) is one of the most common gynecological endocrine diseases in women of reproductive age. Women with PCOS are more likely to develop vitamin D deficiency. Several studies investigated the relationship between serum vitamin D level and LDL-C blood level in woman with PCOS. The results of those clinical trials are controversial, with studies that favored vitamin D effects, oppositely to the others that did not confirm potential LDL-c reduction effect of vitamin D. The aim of this meta-analysis was to investigate the influence of vitamin D supplementation on LDL-C blood level.

Methods: A systematical search of PubMed, Cochrane Library, Web of Science, and Scopus databases were performed for randomized clinical trials considering vitamin D supplementation and LDL-c level reduction, until December, 2018. Random effect model was applied to calculate pooled overall effects.

Results: The results of this meta-analysis demonstrated that vitamin D supplementation failed to significantly reduced LDL-C blood level (WMD = 1.90; 95% CI = −2.20 to 6.00; $P = 0.36$). The significant heterogeneity was detected (Tau2 = 14.33; Chi2 = 16.83; df = 8; $P = 0.03$; I2 = 52%). Funnel plot didn't indicate the presence of publication bias.

Conclusion: In conclusion, vitamin D supplementation cannot reduce the LDL-c level, and therefore we can propose that it can't reduce cardiovascular risk in woman with PCOS. The effect of vitamin D on other cardiovascular risk factors should be investigated.

Key-words: Polycystic ovary syndrome, vitamin D, LDL-C blood level, Meta-analysis

P124-T | Low physical activity increasing atherosclerotic lipidic markers in fatty liver disease patients: perspectives from the ETN 'FOIE GRAS'

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Background: Non-alcoholic fatty liver disease (NAFLD) is a dysmetabolic condition affecting ≈25% of adults, rising atherosclerotic markers such as intima-media thickness (IMT) and epicardial fat (EF). An active lifestyle, including moderate-high physical activity, decreases its progression. We aimed to assess the impact of physical activity in atherosclerotic markers of adult NAFLD patients and young healthy subjects from Apulia, Italy.

Material and methods: Fifty NAFLD adults from a gastrointestinal clinic (M:F = 30:20; mean age 45 ± 1.6 years; range 21-66; 54% obese) were gender-matched with younger controls (M:F = 25:25; mean age 28 ± 1.3 years; range 20-62). Liver steatosis, IMT and EF were assessed ultrasonographically (Noblus® Hitachi, 3.5, 7.5 MHz Probe, Italy; score 0-3). Levels of physical activity were reported by Metabolic Equivalent Tasks (METs; 1MET = 3.5 mL/kg/min of oxygen consumption or 1.5 kcal/kg/h), using the International Physical Activity Questionnaire ('IPAQ', Minetto et al. 2018).

Results: NAFLD patients (mean liver steatosis: 1.6) were heavier than controls (95.5 ± 2.7 vs 64.1 ± 1.8 kg; BMI: 32.2 ± 0.9 vs 22.4 ± 0.6 kg/m², respectively; $P < 0.0001$ for both). Both IMT and EF were thicker in patients than controls (1.0 ± 0.1 vs 0.6 ± 0.0 mm and 8.1 ± 0.4 vs 4.5 ± 0.2 mm, respectively; $P < 0.0001$ for both). Patients with abnormal IMT (≥1.0 mm) and abnormal EF (≥5.0 mm) were 34% (n = 17), whereas 92% (n = 46) presented abnormal EF. None of the controls had abnormal IMT, but 44% (n = 22) had slightly increased EF. Patients were more sedentary (≥900 METs/week) than controls (58% vs 18%, respectively; $P = 0.001$). Both groups preferred light physical activity (≈3.0 METs; 83% overall), with controls exercising longer at this intensity than patients (1310 ± 147 vs 579 ± 127 METs/week, respectively; $P < 0.0001$).

Conclusion: An increased risk for atherosclerosis in NAFLD patient is rising in Southern Italy. Physical activity remains low, worsening the fatty liver condition, regardless of healthy lifestyle indications from physicians.

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P125-T | Dynamic assessment of liver function in subjects with limited hepatic function: The ^{13}C-Methacetin Breath Test for hepatocyte microsomal performance

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Background: Liver steatosis is an emerging health problem worldwide due to the rising prevalence of obesity and metabolic syndrome. NAFLD is a predisposing condition to the necro-inflammatory steatohepatitis (NASH), liver fibrosis, cirrhosis and hepatocellular carcinoma. The non-invasive assessment of liver function in NAFLD patients may improve defining prognosis and management.

Material and methods: Twenty-three subjects with limited hepatic function (LHF, M:F = 15:8, age 45 ± 2.4 years, BMI: 31.6 ± 1.1 kg/m^2, 57% obese) and 16 controls (M:F = 7:9, age 30 ± 2.9 years, BMI: 23.0 ± 1.1 kg/m^2) were enrolled. Liver steatosis by ultrasonography (Noblus Hitachi, Japan; score 0-3) and liver fibrosis by ARFI (Acoustic Radiation Force Impulse by GE Healthcare, USA; score F0-F4) were assessed. Liver blood flow and hepatocyte microsomal function were measured by ^{13}C-methacetin breath test. $^{13}CO_2$ production in expired air was measured by Helifan IR-Mass spectrometer (AB Analitica, Italy) comparing delta-over-baseline (DOB, 15 and 30 minutes), and complete percent dose recovery (CPDR, 30 minutes).

Results: In LHF subjects, liver steatosis degree was S1, S2 and S3 (70%, 22% and 8% respectively) and liver fibrosis degree was F0, F1, F2, F3 and F4 (52%, 13%, 22%, 4% and 8% respectively). Steato-fibrosis was present in 48% of LHF subjects. Portal delivery of substrate (DOB15' < 14.5) was delayed in 57% of LHF subjects (6/13 with steato-fibrosis), showing a trend when compared to controls ($P = 0.051$). Only 19% of controls had a delayed response in DOB15', and only four LHF subjects (17%) presented defective microsomal function (CPDR30' < 8.1).

Conclusion: In overweight-obese subjects with mild steatosis, hepatocyte microsomal function was related to portal blood flow and tended to correlate inversely with fibrosis, indicating decreased liver function, reduced portal flow and decreased substrate extraction/metabolization. Functional breath tests might provide additional about the microsomal functionality of the liver.

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P126-T | Accumulating activated platelets and microparticles in the blood of autoimmune patients reflect NETs generation

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Activated platelets express phagocytic tags on their membrane which restrict their hemostatic and inflammatory potential. Uncleared platelets generate microparticles (PDuPs) that express HMGB1. Membrane-associated HMGB1 acts on neutrophils influencing their metabolism, extending their survival and prompting the Neutrophil Extracellular Traps (NETs) formation, which in turn contribute to tissue inflammation and injury in diseases where the phagocytic clearance is jeopardized, such as systemic lupus erythematosus (SLE).

The internalization of platelets by neutrophils, counts of activated platelets and of PDuPs expressing HMGB1 and the concentration of plasma NETs plasma were analyzed in 95 patients with systemic diseases, such as SLE, systemic sclerosis (SSc), granulomatosis with polyangiitis (GPA) and rheumatoid arthritis (RA) and 30 age- and sex-matched healthy subjects. The effect of phagocytosis on NETs formation was verified in vitro. The results were confirmed by verifying NETs formation after in vivo infusion of PDuPs in immune deficient NSG mice.

Activated platelets and PDuPs were significantly more abundant in the blood of patients with SLE, with SSc and with GPA compared with patients with RA or with healthy controls. Accumulation of PDuPs in the blood was inversely correlated with the detection of intracellular platelet in neutrophils. The plasma concentration of NETs byproducts was significantly higher in patients in which activated platelets and PDuPs accumulated. Purified neutrophils lose upon phagocytosis of particulate substrates the ability to generate NETs in vitro when challenged with standard agonists (IL-8, HMGB1). PDuPs injected in NSG mice effectively trigger the formation of NETs in NSG mice.

The array of signals associated to and released by activated platelets influence both locally and systemically the threshold of NET generation in patients with systemic autoimmune diseases. Further studies are needed to determine if and how this pathway is in these patients involved in the persistence of vascular inflammation and in the associated tissue damage.

P127-T | Fetal breathing movements and pulmonary lymphatics function together to prepare the lung for inflation at birth

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Embryonic lungs must be inflated immediately after birth to establish respiration. In addition to pulmonary surfactant, recently we have revealed lymphatic function as a previously unknown mechanical regulator of prenatal lung compliance that prepares the embryonic lung for inflation at birth. It is well-documented that the late gestation embryo performs episodic breathing-like movements called as fetal breathing movements (FBMs), but the physiological importance of these events is not clear. In our current study we aimed to define the physiological role of FBMs in preparation for air inflation.

$Clp1^{K/K}$ embryos were used because these embryos during late gestation develop progressive loss of spinal motor neurons associated with axonal degeneration and denervation of neuromuscular junctions serving as an ideal genetic model to test the possible role of FBMs. Paraffin based histology was performed followed by HE and immunostaining against lung developmental and lymphatic markers. Lymphatic function was assessed by injecting fluorescently labeled macromolecules into the developing lung of Clp1 $^{K/K}$ and littermate control.

We demonstrated that Clp1 newborns show impaired motor function resulting in fatal respiratory failure after birth. Next, we characterized the development of the embryonic lung before air inflation. The alveolar septae are thicker, and the alveolar area is reduced in late gestation embryos lacking FBMs, while the lack of FBMs does not influence molecular lung development. Importantly, pulmonary lymphatic vessels appear to be dilated and the prenatal pulmonary lymphatic function is impaired in embryos lacking FBMs.

Our results have revealed the previously unrecognized role of FBMs in prenatal lung expansion, suggesting that FBMs and prenatal pulmonary lymphatics function together to prepare the developing lung for inflation and gas exchange at birth. Stimulating FBMs during late gestation might be an effective way to reduce the risk of the development of neonatal respiratory failure.

P128-T | Sensitization to allergocomponents in patients with allergy to Birch pollen and oral allergy syndrome (OAS)

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Background: The similarity of the birch pollen and proteins of vegetables and fruit leads to development of cross-reactivity to plant-origin food proteins and OAS and requires the creation of innovative diagnostic algorithms.
Material and methods: 48 patients with allergic rhinitis, bronchial asthma and OAS were examined (patient's history, skin testing). Birch pollen allergocomponents rBet v1, rBet v2; rBet v4, rPhl p1, rPhl p 5b, rPhl p7, rPhl p12 were evaluated by ImmunoCAP Phadia IDM. Food panel included test with extracts F49, F31, F14, F35, F9, F33, F95 (n = 28) and allergocomponents (PR10) Mal d1, Gly m4, Dau c1, Pru ar1 (n = 20).
Results: Sensitization to rBet v1 was detected in 97.9%, to rBet v2, rBet v4 in 13.3%. Increased level of ASIgE was detected for F49—61; F31—36; F14—58; F35—33; F9—4; F33—11; F95—61; Mal d1—85, Gly m4—65, Dau c1—60, Pru ar1—60%. Coincidence of lab results with patient's history was for F49—33.5; Mal d1—42.5; Gly m4—15.9; F35—6.8; F33—4.2%. Sensitization to ≥2 foods was detected for patients with a positive level of ASIgE together to PR10 and profilines (84%, $P < 0.05$). Mono-sensitization to food was more often in patients showed increased level of ASIgE to rBet v1 or rBet v2, rBet v4 ($P < 0.05$).
Conclusion: Use the individual diagnostic panels with the most significant components and extracts of food allergens and working out standardized panels according to local features will be effective for diagnosis OAS in patients with birch pollen allergy.
Work supported by Program of Competitive Growth of KFU.

P129-T | Importance of immunological tests for prevention latex allergy in healthcare workers

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Background: Work in healthcare service, associated with contact to latex products, is a risk factor for developing latex allergy (LA).
Material and methods: Values of total (normal—up to 100 UE/mL) and specific (to latex; normal—up to 0.35 UE/mL) serum IgE were determined in 120 healthcare workers with suspected LA.
Results: Increased level of specific IgE was detected in 57 subjects (47.5%). Values of specific IgE >0.5 UE/mL were detected in subjects with average age 35.5 years and professional experience of 9 years. There was a tendency to increasing values of total IgE in subjects with high level (>0.5 UE/mL) of specific IgE to latex.
Topical skin symptoms were determined in subjects with values of specific IgE 0.35-0.5 UE/mL. Rhinitis and conjunctivitis together with skin symptoms were determined in subjects with values of specific IgE over 0.5 UE/mL. Subjects with normal values of specific IgE showed atopic history less often compared to subjects with increased values of specific IgE to latex.
Relative risk (RR) for increased specific IgE level in subjects with atopic history was 2.53 (95% CI 1.63-3.94; $P < 0.001$) and OR = 5.51 (95% CI 2.52-12.05; $P < 0.001$); EF = 60.5%. These data indicate a high risk of professional reason for LA in subjects with atopic history.
Conclusion: Latex allergy in healthcare workers manifested by skin symptoms and systemic reactions. LA is a most common allergy in healthcare workers with average age 35.5 years and professional experience of 9 years with atopic history (confirmed by high level of total serum IgE; $r = 0.42$, $P = 0.018$). These data can be important for medical expertise before applying for a job in healthcare service.
Work supported by Program of Competitive Growth of KFU.

P130-T | Examination of kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) in patients with chronic kidney disease

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Background: Markers of acute kidney injury such as kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) can also be biomarkers for diagnosis and prediction of the progression of chronic kidney disease (CKD). CKD patients has high morbidity and mortality, so further investigation of the renal function biomarkers is necessary for the timely diagnosis and prediction of renal impairment.

Material and methods: This study included 72 patients with CKD of the calculated glomerular filtration ratio (eGFR) below 60 mL/min, arranged in four stages of the disease, depending on the level of renal damage (stage IIIa- eGFR 45-59 mL/min, stage IIIb—eGFR-30-44 mL/min, stage IV- eGFR 15-29 mL/min, stage V hemodialysis-HD eGFR <15 mL/min). KIM-1 was determined in serum of subjects, while the NGAL was determined in urine.
Results: The KIM-1 values were lower in patients with stage IV of the renal disease compared with the control healthy group ($P < 0.001$) and with moderate renal impairment groups (CKD IIIa vs CKD IV $P < 0.001$; CKD IIIb vs CKD IV $P < 0.05$). A higher value of KIM-1 was measured in hemodialysis patients compared to the CKD stage IV patients ($P < 0.001$). The values of NGAL in hemodialysis patients were far lower than the control group of healthy and other stages of renal disease ($P < 0.001$). A low positive correlation between NGAL and creatinine clearance ($P < 0.01$; coefficient of correlation 0.42) and low negative correlation of NGAL with creatinine and urea serum levels was observed.
Conclusion: No major difference in NGAL values was observed between healthy and CKD stages IIIa, IIIb, and IV. With worsening of renal function, lower values of NGAL were measured. Further investigation of KIM-1 as a potential marker of the terminal stage of CKD is necessary, considering its higher values in the group of dialysis patients.

P131-T | Analysis of arrhythmic patterns by the statistics of reoccurrence times between consecutive ventricular arrhythmic complexes

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Background: Arrhythmia events including ventricular premature complexes (VPCs) are common cardiac rhythm disorders, and their temporal arrangement contains potentially important diagnostic information on the patient's condition.
Materials and methods: The study examined 56 ECG circadian records obtained at the Almazov National Medical Research Centre (St. Petersburg, Russia) containing at least 1000 extrasystole episodes each. The intervals of more than 6 seconds between arrhythmic episodes were considered and their complementary cumulative distribution functions $R(\tau)$ were estimated and cluster analysis has been performed.
Results: All records could be classified into 3 groups, including (1) 21 records with stretched exponential (SE), (2) 29 records with power law (PL) tailed $R(\tau)$ and (3) 6 unclassified cases. Cross-correlation analysis with common clinical indicators revealed that (a) both the average and the maximum heart rate values in the PL group were systematically higher than in the SE group ($P = 0.04$ and $P = 0.02$ respectively); (b) both the total number of VPCs as well as single arrhythmic episodes per circadian cycle in the PL group were systematically higher than in the SE group ($P = 0.04$ and $P = 0.03$ respectively).
Conclusion: Our results indicate that temporal arrangement of arrhythmia events contains potentially important diagnostic information on the cardiac rhythm anomalies and could be used as complementary indicators when studying their alternations with decease and aging.
Funding sources: This research was partially supported by the Ministry of Science and Education of the Russian Federation, project number 2.5475.2017/6.7 and by the President's of Russia Grant Research Council, project number SP-1451.2018.4.

P132-T | The logistic regression model for cardiac remodeling prediction

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Background: The cardiac remodeling often occurs in patients with hypertrophic cardiomyopathy in the presence of obesity. Timely prediction of cardiac remodeling will provide the personalized treatment of these patients and improve their clinical condition.
Materials and methods: We analyzed the database obtained the characteristics of 136 patients with hypertrophic cardiomyopathy. The remodeling was established if the left ventricular posterior wall thickness exceeded 10 mm. We found a set of anthropometric and echocardiographic indicators, significantly related to the presence of remodeling. Then we built a binary logistic regression model to predict the cardiac remodeling and estimated its quality.
Results: The best characteristics of the prediction model were obtained using the following predictors: age group before or after 60 years ($P < 0.001$), patient gender ($P < 0.05$), waist circumference ($P < 0.01$) and ejection fraction ($P < 0.005$). The power of the constructed binary logistic regression model is equal 93.9%. The total percentage of correct predictions is 85.3%. Nagelkerke R Square coefficient is 0.723.
Conclusion: Our results indicate that age of patients is the most significant cardiac remodeling predictor. The waist circumference is more significant than the weight, body mass index, overweight or obesity. Our model may be useful in

predicting cardiac remodeling in patients with hypertrophic cardiomyopathy.

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P133-T | Pro-B-type natriuretic peptide strongly predicts future cardiovascular events in cardiovascular disease patients with type 2 diabetes as well as in those without type 2 diabetes

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Background: Pro-B-type natriuretic peptide (proBNP) is a prognostic biomarker in various patient populations. Its power to predict cardiovascular events in the extremely high risk group of patients with the combination of established cardiovascular disease (CVD) and type 2 diabetes (T2DM) is unclear and is addressed in the present study.

Material and methods: We measured serum proBNP in 900 patients with established CVD including 591 patients with angiographically verified coronary artery disease and 309 patients with sonographically proven peripheral artery disease. Prospectively, we recorded vascular events over 6.3 ± 2.0 years.

Results: At baseline, proBNP was significantly higher in patients with (n = 317) than in those without T2DM (990 ± 2556 vs 742 ± 2328 pg/mL; $P = 0.003$). The cardiovascular event rate was significantly higher among CVD patients with than among those without T2DM (50.5 vs 35.1%; $P < 0.001$). ProBNP significantly predicted the incidence of cardiovascular events after adjustment for age, gender, BMI, smoking, systolic and diastolic blood pressure, LDL cholesterol, HDL cholesterol and the eGFR both in patients with T2DM (standardized adjusted HR 1.48 [1.28-1.73]; $P < 0.001$) and in subjects without T2DM (HR 1.33 [1.20-1.47]; $P < 0.001$).

Conclusion: We conclude that serum proBNP in patients with established CVD predicts future cardiovascular events independently of established cardiovascular risk factors both among those with as well as among those without T2DM.

P134-T | Pulmonary thromboembolism initially misdiagnosed as acute coronary syndrome

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Background: Pulmonary thromboembolism (PT) sometime resembles ACS as they have common symptoms. Patients who survived PT present signs which can be incorporated for better understanding of PT genesis and differential diagnosis between PT and ACS.

Material and methods: Twenty-three patients with mean age of 59 years with ACS where included into the study. PT was diagnosed according CT scanner and D-dimer test.

Results: Number of cases increased with increase of age. In 91% of cases overweight was discovered: BMI was 28 in men and 34 kg/sq.m in women. Time of admission did not show relation to morning peak of catecholamine concentration in blood. Patients were admitted in 1 to 4 days after onset of disease, stayed for 12 days in average, with 3 days in intensive care. Longer stay was related to tachycardia average 110 per minute and enlarged cardiac shadow on chest X-ray, which can be related to enlarged RV. On echocardiography left heart chambers were not considerably changed in size, but right chambers were enlarged in almost 80% of patients. LV function was preserved. Concomitant diseases were equal in men and women, but constitutional risk factors were more expressed in women. Most frequent sign was dyspnea observed in 91%, weakness and precordial pain were present in half of patients, cyanosis was observed only in 5% of patients. Precordial pain was usually marked but was not related to ECG abnormality. Troponin I concentration was increased in 1 patient with DCM. Decrease in BP reached level of shock grade I in quarter of all examined patients.

Conclusion: Echocardiography and typically present clinical signs are highly significant in diagnosis of PT.

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P135-T | Exploring the potential of salivary and blood immune profile makers to explain physical frailty in institutionalized older women

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Background: Early identification of older populations at increased risk of frailty using biochemical approaches is potentially important for screening accuracy and prevention of early death. The aim of this study was to assess how different blood and salivary biomarkers could be applied to accurately explain frailty and study the relationship between immune markers and independent components of frailty.

Materials and methods: A group of 139 institutionalized-dwelling women, aged 75 years and more, were assessed for biosocial, general health status and physical frailty using the five Fried's physical frailty components. The sample divided into frail, pre-frail and non-frail subgroups. Different biochemical makers including pro and anti-inflammatory cytokines, sex steroid hormones, salivary anti-microbial proteins and blood cells count were quantified. The comparison between the frail subgroups was performed using ANOVA or Kruskal-Wallis. Association between sub-groups and category variables was assessed using Chi-square tests (Monte Carlo simulations were used when applicable). The univariate diagnostic value of each marker was determined using ROC (receiver operating characteristics) analysis. For each model, a 95% confidence interval from percentiles for the area under the curve (AUC) was determined. Relationships between physical frailty components and biomarkers were assessed using linear regression or through logistic regression.

Results: Results indicate that salivary α-amylase was the biomarker that better explained the frail condition and also the one that better associated with the independent components of frailty. Inflammatory markers and blood counts (MCH, IL-10, IL-1beta, TNF-alpha and cortisol levels) also associated with the frailty components of Weakness and Exhaustion.

Conclusions: These results have uncovered a new salivary marker (alpha-amylase) that may be used as a screening tool. Future research needs to investigate the causal-effect association between immune makers and frailty and further explore the role of neuroendocrine or musculoskeletal biomarkers in frailty.

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P137-T | Risk factors of subclinical atherosclerosis beyond cholesterol

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Introduction: Heart diseases are despite the advances in prevention and treatment, still the most important factor of mortality. The aim of this study was to determine the relationship between selected additional risk factors and functional & structural vascular changes in subclinical atherosclerosis patients.

Methods: 179 healthy patients were divided into 3 groups based on BMI respectively based on the degree of carotid artery atherosclerotic changes: negat. group; cIMT >75‰; asymptomatic plaque group. PCSK9, H-FABP, leptin, hsCRP and FFA were measured. Vascular changes (cIMT; PWV; AI; Beta), liver steatosis (hepatorenal index—HRI) and epicardial fat thickness were quantified by ultrasound.

Results: Significant increase of PCSK9, CD163, leptin and FFA in increased BMI groups were found. Significant correlation of EFT and cIMT, respectively HRI was found. Significant correlation of PCSK9 concentrations and BMI, respectively EFT, PWV and Beta was found. Significant increase of PCSK9 was detected between healthy group and group with cIMT >75‰, however no significant increase was detected between cIMT >75‰ and negative group. Positive linear correlation of PCSK9 concentration and vascular changes were found (cIMT,AI,β,PWV, each $P < 0.001$), however after reanalysis in groups, this correlation persisted only in group with borderline values. Besides that, significant linear correlation was found between the PCSK9 concentrations and lipid parameters (LDL $P < 0.001$; Lpa $P = 0.036$). However, no significant correlation was found with hsCRP ($P = 0.77$). Values of CD163 significantly linearly correlated with Beta, PWV, cIMT and leptin with HRI, EFT, PWV and cIMT.

Conclusion: Important regulatory role of PCSK9 in the early development of vascular changes is suggested. Monitoring of hsCRP and CD163 showed us that patients with dysregulation of fat tissue (with inflammatory reactions) could

have these changes mediated by PCSK9&FFA. According to these results it is suggested that the best way to identify the patients with high cardiovascular risk is to monitor the EFT proliferation and increased levels of PCSK9, which corresponded best with subclinical vascular findings.

P139-T | Stimulation of hydrogen sulfide synthesis improves endothelium dependent vasorelaxation and restores of cNOS activity in old rat aorta

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Background: Hydrogen sulfide (H_2S) is considered to be the youngest representative of the family of gas transmitters. It is known that this gas mediator provides cardio- and vasculoprotective effects, in particular in myocardial infarction, arterial hypertension, etc. The aim of the current work was to study the effect of H_2S-synthesizing enzymes cofactor pyridoxal-5-phosphate (PLP) on the H_2S content, cNOS activity and NO synthesis and endothelium-depended aorta smooth muscles (SM) relaxation in old rats.

Materials and methods: We used Wistar male rats divided into 3 groups: adult (6 months), old (24 months) and old+PLP. PLP was administered per os in a dose of 0.7 mg/kg once a day for 2 weeks. Muscle contractile activity of the aorta rings was measured with tensiometry in a chamber at 37°C. Additionally, the content of H_2S, cNOS and iNOS activity, was measured in aortic tissues.

Results: Our results show that H_2S content and cNOS activity was 1.6 times and 3 times lower respectively, in old rat aorta comparing to adult ones. However, PLP administration induced the increase of endogenous H_2S content and cNOS activity in 2.4 times and 2.1 times respectively, compared with those in older animals. Additionally, iNOS activity was 3.8 times higher in old rat, but the administration of PLP caused decreasing of this parameter in 1.9 times. Endothelium-depended relaxation of SM was greatly impaired in old rats: the index of Ach-induced relaxation was $18.4 \pm 4.1\%$ vs $66.5 \pm 6.4\%$ in adult. After PLP administration the index of aorta relaxation was $47.7 \pm 4.8\%$ that indicates at least partial restoration of endothelium-depended relaxation of aortic SM.

Conclusions: Thus, PLP administration might be used for stimulation of endogenous H_2S synthesis, restoration of cNOS activity and normalization of endothelium-dependent relaxation of the SM of the aorta which can be an important regulatory factor in the development of cardiovascular disease.

P141-T | Plasma ApoE elevations are associated with NAFLD: The PREVEND study

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Background: Non-alcoholic fatty liver disease (NAFLD) is featured by increased plasma triglycerides, consequently to very low density lipoproteins (VLDL) elevations, but the extent to which plasma apolipoprotein E (ApoE) is elevated in NAFLD is unclear. Here we determined the relationship of plasma ApoE and its genotype with NAFLD.

Materials and methods: Plasma ApoE and genotypes were determined in 6762 participants of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort. Fatty Liver Index (FLI) ≥60 was used as a proxy of NAFLD.

Results: In total 1834 participants had a FLI ≥60, which coincided with increased prevalence of type 2 diabetes mellitus, metabolic syndrome, (central) obesity, elevated triglycerides, higher non-high density lipoprotein cholesterol and apolipoprotein B (all $P < 0.001$). ApoE was increased in subjects with an elevated FLI compared with subjects with a normal FLI (0.048 vs 0.036 mmol/L, $P < 0.001$). In multivariable linear regression analysis, plasma ApoE levels were positively associated with an elevated FLI when taking account of ApoE genotypes and other clinical and laboratory covariates (fully adjusted model: $\beta = 0.201$, $P < 0.001$). In a sensitivity analysis (n = 4730), which excluded subjects with positive cardiovascular history, impaired estimated glomerular filtration rate, elevated urinary albumin excretion and drug use, plasma ApoE was also independently associated with elevated FLI ($\beta = 0.221$, $P < 0.001$). Stratified analysis for ApoE genotypes (ApoE ε3ε3 homozygotes, ApoE ε2 carriers, and ApoE ε3ε4 and ε4ε4 carriers combined), showed independent positive associations of plasma ApoE levels with an elevated FLI (all $P < 0.001$).

Conclusion: Increased plasma ApoE levels are associated with NAFLD, even when taking account of different ApoE genotypes. Increased plasma ApoE may contribute to altered VLDL metabolism in NAFLD.

P142-T | Content and stability of aorta atherosclerotic plaques: comparing the results of clinical and physicochemical characterizations for operational tissues and synthesized calcium phosphates

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Background: Vascular calcification with ageing is a complex process that exhibits a number of characteristic morphology patterns in atherosclerotic plaques (ASP). Mechanisms connected with ASP formation and calcification as well reliable factors/markers of their stability are a matter of controversy demanding various research tools and approaches for their investigation.

Material and methods: Plaque tissues of aorta walls from 80 male patients (56 y.o.) after carotid endarterectomy operations were investigated histologically, with ultrasound (LOGIQ E9, Aplio XG), scanning electron microscopy (SEM, Merlin), electron dispersive spectrometer (EDS, AZtec), X-ray diffraction (XRD, D8-Advance), thermogravimetry/ scanning calorimetry (TG/DSC/MS STA-449 Jupiter), pulsed electron paramagnetic resonance at low and high magnetic fields (EPR, Bruker E-680). The results are compared with the synthesized by different ways calcium phosphates (CaP) - hydroxyapatite (HA), tricalcium phosphate (TCP), octacalcium phosphate (OCP) doped by various divalent cations in amount 0-20 mol%

Results: Ca, P, O, Na, Mg, Cl, K, Cu elements were detected in ASP. From the SEM/EDS/XRD the presence of only HA in the calcified (Ca/P >1.0) and other CaP (close to TCP) in samples with Ca/P <0.6 was found. No traces of Cu^{2+} and Fe^{3+} ions but Mn^{2+} and radiation induced CO_2- radicals were detected and identified by EPR. Their EPR spectral and relaxation characteristics depends on the calcification degree, location (cap, shoulders or core of ASP), ASP stability. Correlations ($P < 0.05$) between the CO_2- concentration and calcification, relaxation characteristics of Mn^{2+} and ASP stability were found.

Conclusions: ASP calcification can be considered as a progressive destabilizing pathological process The degree of calcification is not an obligatory criterion for plaque stabilization. No other CaP besides HA in ASP and wall tissues were reliably detected. Presence and properties of paramagnetic ions can serve as markers of plaque calcification and instability.

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P143-T | Senolytics: a therapeutic solution for vascular aging?

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Cardiovascular diseases (CVDs) are one of the major causes of morbidity in the increasing aging population of developed countries. CVDs are characterized by the accumulation of senescent cells in the aged vascular tissue. Senescent cells have a typical secretory phenotype (SASP), constituted mainly by matrix remodelling enzymes and inflammatory factors. Increased senescent cell and SASP overload can lead to organ damage, decreased stress resistance and increased risk of contracting age-related diseases (A. Hernandez-Segura et al., 2017) (T. Tchkonia et al., 2013). The clearance of senescent cells from blood vessels using genetic or pharmaceutical tools lead to a decrease in vascular aging and an increase of both animal health and lifespan (B. G. Childs, et al., 2016). Yet, to prevent drug side effects it is important to identify senolytics with the highest pharmacological activity. Arterial endothelial cells (ECs) are major players in the initiation, development and disruption of vascular ageing. Therefore, we tested several known senolytic compounds and studied their impact in clearing senescent ECs. Quercetin and Fisetin induced very low toxicity to arterial ECs after a 24 hours treatment. Navitoclax showed the highest senolytic potential, clearing more than 80% of senescent artery ECs after a treatment of only 8 hours and decreasing non-senescent cell viability by less than 20%. Our results show that Navitoclax might be an interesting therapeutic solution for vascular ageing.

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P144-T | Protein complexes of gold nanoparticles capped by amphiphilic calixresorcinarenes for the biomedical applications

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Background: Gold nanoparticles (AuNPs) have a large number of advantages to be used in the cell based therapy for biomedical applications since they possess unique electron-optical properties, small size, biocompatibility, thermal stability, non-toxicity and ability to bind target substrates on their surface, including biomolecules, e.g. proteins. Modification of AuNPs by water-soluble supramolecular macrocycles, such as calixarenes or calixresorcinarenes, seems to be of current interest for protein binding due to the opportunity of such ligands gladly interact with protein and act as modifiers of protein surface and change its state, leading to biological response.

Material and methods: We carried out synthesis of AuNPs (score = 25 nm) using tetramethylensulfonated tetrapentylcalix[4]resorcinarene both as reducing and stabilizing agent in an aqueous solution at 25°C. Obtained nanoparticles Au@C5S were characterized by spectrophotometry, TEM, DLS, IR and TGA. Investigation of Au@C5S interactions with bovine serum albumin (BSA) was carried out by fluorimetry titration and synchronous fluorescence methods.

Results: Au@C5S form stable complexes with protein. Mechanism of BSA interaction with Au@C5S was investigated: quenching (KSV) and binding (Kas) constants, number of binding sites (n), and thermodynamic interaction parameters (ΔG, ΔH, ΔS) were calculated. The process of protein binding by macrocycle and modified AuNPs is exothermic and spontaneous, and van der Waals and hydrogen bond interactions make a major contribution into the binding. Binding of BSA with macrocycle influences on the microenvironment of tryptophan residues in protein molecule.

Conclusion: Interaction of Au@C5S with BSA leads both to the changes in protein structure and in physicochemical characteristics of AuNPs, which is a promising aspect in usage of such systems for transport of protein molecules or their visualization-detection in biological media.

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P145-T | Design of molecular carriers based on sulfonated viologen cavitand for redox-induced substrate delivery

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Background: Imbalance and increased formation of glutathione is a characteristic feature of various pathologies, such as cancer, ischemia, cystic fibrosis, HIV. Therefore, carriers that respond to an increased concentration of glutathione and dynamically release drugs are very promising in the cell based therapy. Our aim was creation of glutathione responsive carrier of water insoluble drugs. For this, the carrier with a redox-sensitive hydrophobic core and a multiply charged shell was created. A hydrophobic core for solubilization of poorly water-soluble drugs consists of disulfide bonds and decomposes with glutathione excess. A multiply charged shell includes sulfonated viologen cavitands for effective interaction with the surface of cells. Sulfonate group reduces the toxicity and bioactivity of viologen, decreases the carrier agglomeration and improves its diffusion.

Material and methods: The carrier (p(SVCA-SS)) was obtained by the microemulsion polymerization using diallyl disulfide and viologen cavitand SVCA. A complex of physico-chemical methods were used for the characterization the structure and properties of p(SVCA-SS). The drug encapsulation and yield were controlled by fluorescence spectroscopy.

Results: p(SVCA-SS) is stable in water, buffer solutions and blood plasma. It is practically non-toxic. Blood hemolysis in presence of p(SVCA-SS) does not exceed 4%. A study of cytotoxicity showed that cell viability is about 70% in the presence of 2 mg/mL of p(SVCA-SS). Its capacity for non-polar dyes (pyrene, fluorescein) is about 10% by weight. The decomposition of p(SVCA-SS) and full dyes yield occurs within 10 minutes at the high concentrations of glutathione (8 mmol/L).

Conclusions: An effective redox responsive carrier for drugs was obtained. The dissociation and drug release occurs with an glutathione excess.

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P146-T | Effect of intranasal administration of mesenchymal stem cells on the approximate motor activity of rats after simulation of ischemic stroke

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The periventricular zone of the lateral ventricles, the hippocampus and the olfactory bulbs are at least three areas containing nerve stem cells in the brain of adult rodents. In physiological conditions the neuroblasts, generated by neural stem cells in these areas of the brain, migrate to areas of the brain, which required the intensive formation of new neural networks, for example, in process of memorizing. After a stroke, neuroblasts migrate to the area of neurodestruction. These findings are a compelling argument for further research to develop new treatments by enhancing endogenous neurogenesis in brain injury.

Therefore the aim of our study was to investigate the effect of intranasal mesenchymal stem cell (MSC) administration on the approximate motor activity of rats after modelling ischemic stroke. The ligation of common carotid arteries under anesthesia was performed in male rats of the Wistar line. Parameters of approximate motor activity of rats were evaluated before operation, 3 and 7 days after. There were two experimental series: with administration of MSC and without. There were not observed significant changes of the pattern of approximate-motor activity on the third and seventh days in second group of animals related to intact animals. Thus, the administration of MSC in the acute period after operation was accompanied by faster recovery of motor activity in experimental animals.

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P147-T | The role of ATP-sensitive potassium channels and nitric oxide in the brain stroke

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Activation of the K+ATP-channels is the main component of the response in the models of preconditioning. Its activation in the mitochondrial inner membrane is associated with the prevention of the mitochondrial calcium overload. The mitochondrial pool of calcium plays leading role in the development of the preconditioning. The role of the nitric oxide (NO) in the development of ischemic cell damage mechanisms is equally important. The nature of NO action depends on the intensity of its production, location and the state of the surrounding tissue.

The aim of this study was to investigate the relationship between K+ATP-channels and NO, as well as a comparison of the obtained data with the molecular mechanisms of mitochondria. We found that the expression of the mitochondrial K+ATP-channels was a two-fold decreased in the nervous tissue and the intensity of the S- nitrosylation and nitration of protein was decreased 24 hours after the ischemic preconditioning in the rats. Pharmacological preconditioning with K+ATP-channels activator diazoxide led to a 30% reduction in the concentration of free NO after the simulation of an ischemic stroke, after 9 and 72 hours. We suggested that obtained result linked with the restructuring of the tissue energy metabolism, namely the provision of catalytic sites in the mitochondria and the increased elimination of NO, which prevented the decrease in the cell sensitivity to oxygen during the subsequent period of severe ischemia.

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P148-T | Use of methylprednisolone in combination with polymer conjugates under local delivery conditions for spinal cord injury

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Many methods for treating spinal cord injuries come from the laboratories and are transferred to clinical trials. Many of them are applied as soon as possible after trauma with the hope of weakening secondary damage and maximum preservation of the nervous tissue. Therefore, at the moment of the science development there is an interest in using polymeric systems as systems for delivering drugs to the spinal cord with trauma. The contusion injury was applied at the Th8 level by the modified A. Allen technique. Epidural electrodes were chronically implanted into the studied segments and muscular responses were induced to stimulate the spinal cord. The responses of the examined muscles were recorded before the operation, immediately after the operation and every hour for 6 hours after a contusion injury. The results showed that the soleus and gastrocnemius muscle have similar changes in amplitude characteristics but the soleus is more sensitive to

the immobilization regimen than the gastrocnemius muscle. In the early phase of traumatic spinal cord disease, epidural stimulation changes the functional state of the spinal neurons which is expressed by suppressing the direct motor response of the soleus muscle, increasing the reflex excitability of the motor centers of the calf muscles and inhibiting polysynaptic responses. Infusion of methylprednisolone according to the standard therapeutic protocol leads to an increase in the motor response of the calf muscles of the rat and suppression of the reflex excitability of the motor centers. The application of the polymer complex with methylprednisolone after a concussion of the spinal cord has a comparable effect to methylprednisolone on the centers of the calf muscles of the rat which persists for 6 hours, which confirms the delivery of methylprednisolone into the nerve tissue. This work was supported by the Russian Foundation for Basic Research under grant №18-315-00267.

P149-T | Upregulation of let-7 microrna in mouse models of Machado-Joseph disease ameliorates neuropathology and motor phenotype

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Background: Machado-Joseph disease or spinocerebellar ataxia type 3 (MJD/SCA3) is a genetic neurodegenerative disorder associated with expansion of the number of CAGs in the coding region of the MJD1 gene, which translates into an expanded polyglutamine tract within ataxin-3 protein. MJD patients have severe clinical manifestations and premature death and there is no treatment available to modify disease progression. We and others provided evidence that autophagy impairments contribute to MJD pathogenesis. Recently, we also brought evidence that the let-7 microRNA is a key regulator of autophagy with particular relevance in polyglutamine disorders. This work aimed at investigating let-7 potential as a new therapeutic approach in lentiviral (LV)-based and in transgenic (Tg) mouse models of MJD.

Materials and methods: Injection of LVs encoding let-7 or mir-scrambled (scr) was performed into the striatum of a LV MJD mouse model. At 4 weeks post-injection, the striatum was collected and processed for microRNA, protein and immunohistochemical analysis. Balance and motor coordination were assessed in Tg MJD mice up to 12 weeks post-injection of LV encoding let-7 or mir-scr into the cerebella and after that brains were processed for immunohistochemistry.

Results: Injection of LV vectors encoding let-7 into the striatum resulted in increased levels of LC3-II protein, in a robust and significant reduction in the number of ubiquitin-positive inclusions, as well as, in a significant reduction of neuronal dysfunction in a LV-based MJD model. Moreover, Let-7-treated Tg MJD mice exhibited a better performance in rotarod, swimming pool, and beam walking tests. Likewise, a significantly larger cerebellar layers thickness was observed in the let-7 treated group, suggesting prevention of neurodegeneration.

Conclusions: All in all, let-7 activates autophagy, decreases aggregation and neuronal dysfunction in the mammalian brain and ameliorates MJD motor deficits. Therefore, autophagy activation mediated by let-7 may represent a new promising therapeutic approach for MJD.

P151-T | Multiplex analysis of conditioned medium of mesenchymal stem cells with IL2 overexpression

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Background: Interleukin 2 (IL2) is FDA approved agent for cancer treatment. High dose IL2 therapy can induce durable complete responses in metastatic melanoma and renal cell carcinoma patients. However, systemic administration of high dose IL2 is associated with severe adverse effects. The use of mesenchymal stem cells (MSCs) for directed delivery toward tumor sites can reduce the toxicity. However, IL2 overexpression can lead to other cytokine secretion changes, so MSCs-IL2 cytokine profile should be investigated.

Materials and methods: Human MSCs were isolated from adipose tissue. Cells were largely positive for mesenchymal stem cell surface markers. MSCs were transduced with recombinant lentiviral vectors encoding IL2 and blue fluorescent protein (BFP). Transgene expression was confirmed by quantitative PCR and western blot analysis. Conditioned medium (CM) was collected after 24, 48 and 72 hours of cultivation. Human Chemokine 40-plex Panel (Bio-Rad, USA) was used to analyze CM samples according to the manufacturer's recommendations.

Results and conclusion: After 72 hours of cultivation, IL2 concentration in CM was 2.51 ± 0.0 pg/mL in native hADSCs, 2.34 ± 0.24 pg/mL in hADSCs-BFP and

4322.86 ± 504.64 pg/mL in hADSCs-IL2 sample. A slight decrease (1.5-2-fold) in the secretion of a number of pro-tumor cytokines, such as CX3CL1, CXCL6, IL8, CCL13, CCL15 и CCL20 was observed in hADSCs-IL2 sample. However, a 1.5-fold increase in TNFa secretion in the hADSCs-IL2 sample compared with native hADSCs and hADSCs-BFP was detected.

Slight decrease in secretion of pro-tumor cytokines was observed. However, an increase in TNFa secretion in hADSCs-IL2 cells can contribute to establishing and/or maintenance of low pro-oncogenic levels of TNFa in the tumor microenvironment. Thus, further investigation of hADSCs-IL2 biosafety is required.

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P152-T | Evaluation of the effectiveness of the introduction of methylprednisolone into the spinal cord as part of amphiphilic polymer L6M

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Glucocorticoids are one of the most frequently used therapeutics. They are considered as emergency drugs administered in severe clinical cases, such as sepsis and acute neuronal traumas. The combination of glucocorticoids on the example of methylprednisolone (MP) with amphiphilic polymers is a topical approach for the local treatment of spinal cord injury. In rats with experimental models of contusion injury of the spinal cord, MP-succinate (MPS) is injected in various ways: infusion introduction of the MP intravenously (30 m / kg) and application of the MP in the element and without polymer on the surface of the spinal cord. After 6 hours, the back of the brain was removed. All experiments were performed in compliance with bioethical norms. MP in an organic substrate using gas chromatography / mass spectrometry. Mass spectrometry data was analyzed using MultiQuant 3.0.2 software (AB Sciex). To assess the penetration, the overall level of MPS + MP was evaluated. Our results showed that the MP was not detected in the intact spinal cord and samples containing only polymer. Polymer L6M significantly increases the amount of MP in the spinal cord. This may indicate its effect at the tissue level. You can also expect its effects at the cellular level. In the case of an MP infusion, the data are variable, but it can be said that in combination with the L6M polymer, the concentration of MP is comparable to the concentration during the infusion. The data show that it is possible to achieve local therapeutic concentration of MP and avoid side effects from systemic use.

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P154-T | Towards more mature HPSC-CM via metabolic modulation in 3D culture

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Background: In vitro differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CM) is a crucial process for the use of these cells for therapy and drug discovery. However, cardiomyocytes generated are immature, reminiscent of fetal cardiomyocytes regarding structure, metabolism and function. Here we combined our expertise in metabolic modulation and 3D culture to further enhance cardiac maturation of hPSC-CM.

Materials and methods: Aggregation of hPSC-derived cardiac progenitors was done in a scalable differentiation a protocol yielding highly pure hPSC-CM cultures followed by metabolic maturation. Whole-transcriptome and metabolic flux analyses, transmission electron microscopy, calcium fluxes and viability responses following exposure to cardiotoxic drugs were performed to assess maturation status.

Results and conclusion: When compared to onset maturation, 3D cultures of hPSC-CMs matured with a fatty acid enriched media displayed a down-regulation of glycolysis and lipid biosynthesis related genes and increased expression for tricarboxylic acid cycle, oxidative phosphorylation and mitochondrial genes. Structurally, no differences in sarcomere length or fiber alignment were seen between both matured groups though a significant increase of mitochondrial density was evident in the fatty acid media matured cells. Cell death was observed in a dose-response manner to doxorubicin, with higher sensitivity to toxicity for the fatty acid matured aggregates. This study highlights the relevance of both metabolism and structure for the maturation of hPSC-CM and their sensitivity to cardiotoxic drugs.

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P155-T | The role of impaired platelet contractility in recurrent pregnancy loss

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Background: Hemostatic disorders associated with dysfunction of blood cells are among the most important pathogenic factors in recurrent pregnancy loss (RPL). Platelets are blood cells that have multiple functions, including platelet-driven contraction of blood clots and thrombi that can modulate the course and outcomes of thrombosis. We hypothesized that impaired platelet-mediated contraction of blood clots can increase obstructiveness of microthrombi and aggravate microcirculatory disorders in placenta, thus promoting RPL.

Materials and methods: Here, we studied kinetics of clots contraction in the blood of 35 women with a history of RPL and 25 parous women without obstetric complications in the past. In addition, the prothrombotic status and hypercoagulability were assessed integral hemostasis tests.

Results: In patients with a history of RPL, hypercoagulability was revealed combined with the impaired blood clot contraction, together indicating predisposition to thrombosis. Both the hypercoagulability and defective clot contraction were significantly more pronounced in patients with 3 or more cases of miscarriage compared with patients who had 1 or 2 fetal losses. In addition, a significant inhibition of clot contraction was found in patients with miscarriage occurring after 10 weeks of gestation compared with those who lost a fetus during the earlier period of pregnancy.

Conclusion: These results indicate that chronic hypercoagulability combined with the impaired contraction of blood clots form a premorbid background in patients with a history of RPL. The data confirm a significant pathogenic role of cellular hemostatic disorders in RPL and suggest that the blood clot contraction assay has a predictive value in assessing a risk of recurrent miscarriage. In addition, platelets could be considered as a novel therapeutic target to prevent and/or treat RPL.

Funding sources: Work supported by the Program of Competitive Growth of KFU and grant 18-415-1600004 from the Russian Foundation for Basic Research and the Republic of Tatarstan.

P156-T | Stimulation of platelets with pathogenic immune complexes containing platelet factor 4 leads to calpain activation

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Background: Heparin-induced thrombocytopenia (HIT) is a severe complication of heparin treatment characterized by a low platelet count and a high risk of thrombosis. The disease is promoted by antibodies to the platelet factor 4 (PF4)/heparin complex, leading to platelet activation and death via an unknown mechanism. We studied if PF4-containing immune complexes with pathogenic (KKO) and non-pathogenic (RTO) antibodies can induce platelet apoptosis.

Material and methods: Isolated human platelets were incubated for 15, 60, and 180 minutes with KKO/PF4 or RTO/PF4 complexes as well as with pure PF4 and antibodies followed by determination of intracellular apoptotic markers and calpain activity. The activity of the proteases (calpain and caspase-3) was analyzed by flow cytometry and fluorimetry. Cleavage of procaspase-3 and expression of an anti-apoptotic protein bcl-xl were assessed by Western blot. Platelets incubated with calcium ionophore A23187 were used as a positive control.

Results: Unexpectedly, flow cytometry and fluorimetry revealed no changes in the activity of caspase-3 in platelets treated PF4/KKO or PF4/RTO. The Western blot analysis showed only minor procaspase-3 cleavage and a slight decrease of the level of bcl-xl after 60 minutes of incubation of platelets with KKO/PF4 complexes. By contrast, A23187 caused complete cleavage of ptocaspase-3 and suppression of bcl-xl. Remarkably, treatment of platelets with KKO/PF4 induced significant time-dependent activation of calpain with a maximum activity at 180 minutes comparable with the positive control. Unlike KKO/PF4 complexes, incubation of platelets with non-pathogenic RTO/PF4 complexes or pure KKO and PF4 had negligible effects indistinguishable from untreated platelets.

Conclusions: The results indicate that HIT-pathogenic PF4-containing immune complexes induce direct platelet activation followed by a non-apoptotic death pathway associated with calpain activation. This mechanism, in addition to other pathogenic factors, may underlie low blood platelet count n HIT.

Funding sources: Work supported by the Program of Competitive Growth at KFU.

P157-T | Soluble and platelet-bound p-selectin in the blood of patients with systemic lupus erythematosus

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multi-organ inflammation and a high titer of various autoantibodies, including anti-DNA antibodies. SLE is often complicated with venous and arterial thrombosis that may be due to continuous immune platelet activation in the circulating blood. Activated platelets express and secrete P-selectin, an adhesion molecule used as a marker of platelet activation. Here, we analyzed if there is association between levels of soluble P-selectin, platelet-bound P-selectin and anti-DNA antibodies in the blood of SLE patients.

Material and methods: ELISA was used to measure soluble P-selectin in blood plasma and anti-DNA antibodies in blood serum. Platelet-bound P-selectin was measured in platelet-rich plasma with flow cytometry using fluorescently labeled anti-human-CD62P antibodies. Data were analyzed using a Spearman's rank correlation test.

Results: A mean level of soluble P-selectin in SLE patients (104 ± 19 ng/mL, $n = 15$) was significantly higher than in healthy donors (57 ± 6 ng/mL, $n = 10$, $P < 0.05$). An average titer of anti-DNA antibodies was also elevated in SLE patients (85 ± 22 U/mL vs < 10 U/mL reference range). Platelet-bound P-selectin correlated inversely with soluble P-selectin ($R = -0.59$, $P < 0.05$), indicating chronic platelet activation followed by release of P-selectin from the platelet plasma membrane. A titer of anti-DNA antibodies correlated inversely with the expression levels of platelet-bound P-selectin ($R = -0.69$, $P < 0.01$) and correlated directly with the levels of soluble P-selectin ($R = 0.86$, $P < 0.01$), suggesting immune platelet activation by DNA-containing immune complexes.

Conclusions: The data indicate continuous platelet activation in the blood of SLE patients associated with a high titer of anti-DNA antibodies. In response to the immune activation, platelets express P-selectin from alpha-granules to the platelet surface followed by solubilization of P-selectin in the blood. The chronic immune platelet activation may comprise a prothrombotic mechanism in SLE patients.

Funding source: Program for Competitive Growth at KFU and RFBR grant №19-015-00075.

P158-T | NIR-light dependent delivery of Cre recombinase: an optogenetic tool for DNA modulation in the brain

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Background: Modulation of neural cells has the potential to treat brain disorders. The delivery of genome-editing enzymes as Cre recombinase is a powerful tool that may be used to manipulate cell activity. Spatial and temporal control of such platforms is important to reduce side effects and to study biological aspects that are otherwise difficult to understand. Nanoparticles that release Cre with spatio-temporal control have been described [1]; however, they present low recombination efficiency and in vivo activity remains to be demonstrated. Here, we present a nanoformulation capable of achieving DNA recombination deep within the brain. The formulation was validated in vivo for the modulation of endogenous neural stem cells (NSCs) and for the control animal behavior when administered to ventral tegmental area (VTA).

Materials and methods: We have designed delivery system based on upconversion nanoparticles that respond to near infra-red (NIR) light to intracellularly release Cre (CRE-UCNPs). A reporter cell line was used to access cytotoxicity and delivery. Recombination efficiency in the subventricular zone (SVZ) NSC niche was evaluated using a transgenic Cre reporter mice. Finally, CRE-UCNPs were administered on the VTA together with CRE-dependent channelrhodopsin virus to test the formulation in a Conditioned Place Preference behavioral assay.

Results: We show the potential of CRE-UCNPs in the internalization of Cre rand its escape from endocytic compartments. We further confirm the specificity of light-induced cargo release, both in vitro and in vivo, with the key achievements of gene edition within the SVZ and VTA regions of the adult brain. Our results point that the level of recombination obtained is sufficient to induce behavioral responses in animals.

Conclusions: This gene editing platform with a spatio-temporal control over Cre activity represents a generalist tool for in vivo NIR light-dependent delivery with high tissue penetration.

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[1]Morales, D.P.,et al., Small(2018):

P159-T | The effect of intercellular interaction of MSC and SH-SY5Y in co-culture on result of cisplatin treatment

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The processes of interaction between tumor microenvironment components are importance for the development of new methods for diagnosis and treatment of cancer. We investigated the interaction of MSCs derived from human bone marrow and human neuroblastoma cells SH-SY5Y in co-culture as well as the effect of cisplatin (CDDP) on cell proliferation, Bcl2, Cav1α, Cav1β, Rac1 mRNA expression and the effect of co-cultivation on cytokine profile. To create the co-culture, cells were mixed at 1:1 ratio. The proliferative activity of co-culture was significantly lower after CDDP-treatment (10 μg/mL), however higher in comparison with treated SH-SY5Y. Cav1α expression level in MSCs after co-culturing demonstrated 2.86-fold decrease, in co-culture+ CDDP: 2.5-fold decrease, MSCs+CDDP: 1.4-fold decrease, in SH-SY5Y after co-culturing+ CDDP: 2.4-fold increase, after CDDP treatment: 3.6-fold increase. Cav1β expression level in MSCs after co-culturing was 3.4-fold lower, after co-culturing+ CDDP: 3.7-fold lower, after CDDP treating: 1.6-fold lower, in SH-SY5Y after co-culturing+ CDDP and CDDP treating: same 4.5-fold increase in comparing with control. Rac1 mRNA was increased 4-fold in MSCs after co-culturing and increased 6-fold after CDDP treatment, SH-SY5Y treated with cisplatin demonstrated 1.6-fold increase. Bcl2 expression in MSCs demonstrated 6-fold increase after co-culture and co-culture+CDDP, after CDDP treated 50-fold decrease, in SH-SY5Y co-cultured and co-cultured+CDDP showed 6.5-fold and 8.5-fold increase respectively, CDDP treated SH-SY5Y showed 4.5-fold increase. After 24 hours of cultivation, high concentrations of IL-6 were observed in co-culture, also concentrations of IL8/CXCL8 and MCP-1/CCL2 showed 4-fold and 2-fold increase. Highest level of MIF was observed 72 hours after co-culture. These results may suggest development of cell-cell interaction between cells and exhibition of pro-oncogenic activity of MSCs in co-culture lead to drug resistance of neuroblastoma cells.

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P160-T | Preparation and analysis of artificial extracellular vesicles from mesenchymal stem cells overexpressing TRAIL, PTEN or IFNA17

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Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells. They have low immunogenicity and exhibit homing behavior toward tumor sites. Thus, MSCs can be used for directed delivery of anti-cancer agents. Cytokines are molecular messengers that allow cells of the immune system to interact with each other and other cells. Numerous cell cultures and animal tumor model studies have shown that cytokines have broad anti-tumor activity. Extracellular vesicles (EVs) transfer parental cell cargo to neighboring cells and thus can be used for the delivery of anticancer therapeutic agents.

Human mesenchymal stem cells were isolated from adipose tissue. Cells were largely positive for mesenchymal stem cell surface markers including CD44, CD90, CD29, CD105 and CD73 and negative for hematopoietic stem cell surface markers. The multipotency of cells was confirmed via differentiation into chondrocytes, osteoblasts and adipocytes. MSCs were transduced with recombinant lentiviral vectors encoding cytokines: tumor necrosis factor ligand superfamily member 10 (TNFSF10, TRAIL), interferon alpha-17 (IFNA17) or tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN). The gene expression was confirmed by quantitative PCR and western blot analysis. Extracellular vesicle release from MSCs was induced by cytochalasin B treatment. Isolated EVs were mostly 50-200 nm in diameter which is comparable with natural exosomes. Interestingly, resulting EVs were positive for CD44, CD90 and CD105 cell surface markers, but CD29 and CD73 expression was significantly decreased (about 10%).

The functionality and antitumour activity of the EVs will be further investigated in various transformed cell cultures in vitro.

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P161-T | Cytokine Secretion Profiling of human umbilical cord blood mononuclear cells transduced with adenoviral vectors carrying therapeutic genes

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Over the last decade insufficient effectiveness of current therapeutic approaches for neurodegenerative diseases has led to elevated interest in alternative therapies. In this connection, application of human umbilical cord blood mononuclear cells (UCB-MC) as cell-carriers for the therapeutic genes may be one of the strategies for prevention the death of neurons in neurological disorders Previously we have shown the positive effect of gene modified UCB-MCs simultaneously overexpressing vascular endothelial growth factor (VEGF), glial cell-line derived neurotrophic factor (GDNF) and neural cell adhesion molecule (NCAM) for treatment of transgene mice with model of amyotrophic lateral sclerosis.

In the presented study we evaluated the ability of UCB-MC simultaneously transduced with Ad5-VEGF165, Ad5-GDNF, and Ad5-NCAM1 to produce anti/proinflammatory cytokines, chemokines and growth factors. Condition medium from gene modified (GM) and non-treated (NT) UCB-MC was examined by Luminex xMAP technology using Luminex 200™ multiplex analyzer and MILLIPLEX MAG magnetic bead-based multi-analyte panel (HCYTMAG-60K-PX41). In GM and NT medium the identical secretion profiles of the analyzed factors (EGF, Exotoxin, TGF-a, G-CSF, GM-CSF, Fractalkine, IFNa2, IFN-g, GRO, IL10, MCP-3, IL12-p40, MDC, PDGF-AA, PDGF-AB/BB, sCD40L, IL-1ra, IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-7, IL-8, IP10, MCP-1, MIP-1a, MIP-1b, RANTES, TNFa) was shown. However the increased secretion of VEGF was revealed only in GM medium.

Thus, the study showed that transduction of UCB-MC with adenoviral vectors does not affect the production of anti- or pro-inflammatory cytokines by the cells in vitro. At the same time gene modified UCB-MC synthesize and secrete recombinant proteins. Our results suggest the safety of the adenoviral transduction of UCB-MC in terms of the endogenous biologically active molecules production and the efficacy of UCB-MC as cell-carriers for delivery of the therapeutic genes.

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P162-T | Functional recovery of mini-pigs with spinal cord injury due to epidural spinal cord electrical stimulation combined with ex vivo triple gene therapy

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The current therapeutic strategy for the functional recovery after spinal cord injury (SCI) includes new biotechnological and electrophysiological methods. In the present research we evaluated the efficacy of triple-gene therapy in a combination with epidural electrical stimulation (ES). Mini-pigs with SCI at Th9 level (n = 2) were intrathecally infused with gene-modified umbilical cord blood mononuclear cells (UCB-MC) simultaneously overexpressing recombinant vascular endothelial growth factor, glial cell-line derived neurotrophic factor and neural cell adhesion molecule. ES (25-35 Hz with 7-25 mA and 0.2 ms pulse duration) was started 2 weeks after neurotrauma and performed above the injury site at C5 level to stimulate neuroregeneration and below at L2 level for excitation of central pattern generators.

Two month after SCI analysis of the joint kinematics during ES revealed that the volume of movement in the ankle joint of treated mini-pigs approached the value of intact animals. Moreover, the Porcine Thoracic Injury Behavioral Scale test found the better score of motor function restoration in animals from therapeutic group.

Electrophysiological study of the soleus muscle estimated 60 days after SCI showed that in the control animals (n = 2), the motor (M) response had several phases, and the reflex (H) response was absent. Important, in the therapeutic group stimulation of the sciatic nerve led to the formation of the standard form of M-and H-responses.

Thus, behavioral tests and electromyography data indicate that UCB-MC-mediated triple gene therapy combined with ES stimulate the processes of neurorehabilitation in mini-pigs with SCI. These results might represent a novel avenue for future research into treating patients with SCI.

This study was supported by the grant of Russian Science Foundation No 16-15-00010. Kazan Federal University was supported by the Russian Government Program of Competitive Growth.

P163-T | Histological study of the post-injured mini-pig spinal cord following gene therapy combined with epidural stimulation

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Currently, there are no reliable methods for effective therapy of the consequences of spinal cord injury (SCI). Our previous work has shown the positive effect of cell-mediated gene therapy in combination with epidural stimulation (ES) on remodeling of the post-injured spinal cord in rat. In this study, we evaluated the efficacy of the triple-gene therapy in a combination with ES on mini-pig spinal cord regeneration after severe contusion.

Four hours after SCI at Th9 level mini-pigs (n = 2) were intrathecally infused with umbilical cord blood mononuclear cells (UCB-MC, 2 × 106/200 μL) transduced with adenoviral vectors carrying therapeutic genes encoding vascular endothelial growth factor, glial cell-line derived neurotrophic factor and neural cell adhesion molecule. ES was started 2 weeks after SCI and performed for 6 weeks. ES were given at a constant current alternatively at C5 level to promote neuroregeneration and L2 level to activate local neural circuits. Control animals (n = 2) were intrathecally injected with saline solution.

Two month after SCI the morphometric analysis of pathological cavities volume in the gray matter below the epicenter injury revealed the better tissue sparing in treated mini-pigs (79.4% ± 0.2%), when compared with control animals (76.0%±0.7%). In the ventral horn of the treated mini-pigs the increased expression of Hsp27 (5.69 ± 1.83 vs 2.77 ± 0.57), lower number of Caspase 3-positive cells (45 ± 3 vs 53 ± 4), and a higher level of synaptophysin (28.96 ± 3.19 vs 12.34 ± 3.60) and PSD95 (18.18 ± 3.34 vs 10.25 ± 2.82) immunoexpression were shown.

Thus, for the first time we report the positive effect of ES combined with UCB-MC-mediated triple gene therapy on morpho-functional recovery of post-injured spinal cord in mini-pigs. Results of this study represent a novel potentially successful approach for SCI treatment.

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P164-T | Extracellular matrix during physiological and pathological cardiac aging: a proteomic study

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Background: Aging is a multifactorial and tissue-specific process involving diverse alterations (Lopez-Otin, Blasco et al. 2013), which is associated with a decline of protein, cell, and organ function. Aging in the cardiovascular system is seen by researchers as the beginning of human aging (Aunan, Watson et al. 2016); however, our understanding of cardiovascular aging is relative scarce. In cardiac tissue, extracellular matrix (ECM) is of outmost importance in the regulation of cellular homeostasis and intracellular signaling, and provides an essential structural support for cardiomyocytes. During aging, ECM leads to functional and structural deterioration of the heart (Sessions and Engler 2016). Unfortunately, it is relatively unknown the effect of aging on human myocardial ECM proteins.

Material and methods: In this study, we have performed proteomic analyses on post-mortem human cardiac tissue. Proteins were extracted from formalin-fixed paraffin embedded (FFPE) heart tissues from old and adult young individuals, and also from Hutchinson-Gilford progeria syndrome patients and quantified through Tandem Mass Tags (TMT)-based quantitative mass spectrometry.

Results: We identified an upregulation of ECM proteins in old human myocardium compare to adult young human heart tissue, mainly in the structural proteins such as collagen type VIII (Endothelial collagen), Elastin and Biglycan. We also identified two significant upregulated proteins, Tissue inhibitor of metalloproteinases 3 and a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAM-TS 4), which are involved in ECM remodelling.

Conclusion: Studies are in progress to compare total proteome of cardiac tissue from old and young mice and evaluate the alterations in ECM proteins using decellularized tissue. However, it has been observed a potentially distinct signature of physiological aging in cardiac ECM proteins and also in decellularized matrixes of old mice compared to young.

Funding source: This work is supported by ERA-CHAIR and Inter-University Doctoral Program in Aging and Chronic Diseases fellowship (PD/BD/106051/2015).

P165-T | New thiadiazole calix[4]arene and thiacalix[4]arene derivatives as DNA compacting agents

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Background: Important challenge in gene therapy is to search efficient and safe gene carries capable of compacting, protecting and delivering nucleic acids into the cell. Calixarenes are versatile macrocycles with opportunity to introduce several appropriate functional fragments to perform multivalent binding with target biomolecules. Their easy synthesis, variety of stereoisomeric forms and low toxicity levels make them really promising molecules for gene delivery applications.

Material and methods: Synthesis of (thia)calix[4]arene derivatives with two or four aminothiadiazole fragments on the lower rim in cone or 1,3-alternate stereoisomeric form is carried out. Obtained macrocycles were found to be water soluble and study of their binding with Calf Thymus DNA (DNA CT) was done using ethidium bromide as fluorescent probe. Size and zeta-potential were measured using dynamic and electrophoretic light scattering.

Results: The obtained thiadiazolyl derivatives of calix- and thiacalix[4]arene are capable of effective interaction with DNA CT. An increase in the number of thiadiazolyl fragments from two to four leads to an increase in the stability constant of the calixarene-DNA complex from 2.1 to 3.6 logarithmic units. It was found that a macrocycle containing four thiadiazolyl fragments in a 1,3-alternate stereoisomeric form is capable of 6-fold compaction of DNA CT, while macrocycle with two thiadiazolyl fragments is capable only of 2-fold compaction.

Conclusion: The obtained thiadiazolyl derivatives of calix- and thiacalix[4]arene are capable of effective interaction with DNA CT. A macrocycle containing four thiadiazolyl fragments is capable of 6-fold compaction of DNA CT, which is interesting from the point of view of creating non-viral transfection systems for gene delivery.

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P166-T | Calix[4]arene stereoisomers with pyrazole pharmacophoric groups: potential cervical carcinoma therapy

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Background: Nitrogen-containing aromatic heterocycles are widely used in cell based therapy due to their ability to bind nucleic acids. Attachment of multiple heterocyclic units to low-toxic macrocyclic scaffold would not only increase the therapeutic dose of the drug, but also increase the stability of such drug delivery system due to the covalent attachment of drug. The aim of this work is to synthesize pyrazole-containing calix[4]arene derivatives and evaluate in vitro its biological activity towards normal and cancer cell lines.

Material and methods: Palladium chloride and copper iodide catalysts were employed for the synthesis of ketoacetylene derivative of calix[4]arene, while hydrazine was used for conversion of the ketoacetylene into pyrazole derivative. Cytotoxicity and hemotoxicity (on erythrocytes) of the pyrazole were determined. Following cell lines were employed: M-Hela, MCF7, A-549, Chang liver, and Wi38. Cell viability was evaluated by Cytell Cell Imaging system using Cell Viability BioApp application.

Results: A pyrazole-containing derivative of calix[4]arene in different stereoisomeric forms was synthesized and studied for cytotoxicity towards normal and cancer cell lines. Hemotoxicity tests showed low toxicity of synthesized calixarenes, while cytotoxicity demonstrated no inhibition of Chang liver and Wi38 cell lines, as well as MCF7 and A-549 carcinoma. In contrast, exposure of M-Hela carcinoma cell lines to the 9 μmol/L pyrazole compound solution resulted in their 50% inhibition.

Conclusions: Pyrazole-containing calix[4]arene in partial cone configuration has shown no toxicity towards normal human cell lines and has displayed cytotoxicity towards M-Hela carcinoma at micromolar concentrations.

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P167-T | A novel pH-responsive drug delivery system on the base of calixresorcinarene - methoxypolyethylene glycol system with dynamic covalent conjugation

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Background: One of the emerging direction of new cell based therapy systems creation is design of drug delivery compositions carrying functional fragments conjugated via dynamic covalent bonds. Conjugation of macrocyclic molecules with biocompatible polymer fragments can discover new opportunities in the construction of drug delivery system with convenient topography and improved encapsulation properties as well as the releasing profile. The aim of our work was to obtain low-toxic calixresorcinarene derivative bearing the fragments of biocompatible polymer attached via acylhydrazone bonds.

Material and methods: Methoxy-PEG-550 was used to attach into calixarene platform via acylhydrazone bond formation. Calixresorcinarene - methoxypolyethylene glycol (mPEG) conjugate was synthesized by step-by-step functionalization of tetraundecylcalixresorcinarene. The cytotoxicity and hemotoxicity of the conjugate and it degradation product were determined. Cell viability of Chang liver cell was evaluated by means of multifunctional system Cytell Cell Imaging using Cell Viability BioApp application. Also the hemolytic activity against human red blood cells was tested. The micellization of the conjugate, the encapsulation of drugs and their release under low pH are studied by DLS, TEM, UV-VIS, and fluorimetry methods.

Results: A pH-responsive drug carrier based on tetraundecylcalixresorcinarene - mPEG conjugate bearing pH-responsive acylhydrazone bonds was synthesized and used for encapsulation of anthracycline antitumor antibiotic Doxorubicin and photosensitizer Methylene Blue. The pH-controlled drug release was studied. The cyto- and hemotoxicity tests demonstrated the low toxicity of the conjugate and it degradation product.

Conclusions: A novel calixresorcinarene-mPEG conjugate was successfully created and in vitro tested as low-toxic pH-responsive drug delivery system.

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P169-T | Endothelial dysfunction in the context of heart failure with preserved ejection fraction

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Heart failure, a progressive syndrome linked to ageing, affects 26 million individuals worldwide (Savarese, G. et al. 2017). Half of this patients have preserved ejection fraction (HFpEF) and this number is increasing (Dhingra, A. et al. 2014). Pathophysiology of HFpEF has been proposed as result of high prevalence of comorbidities that induce a systemic pro-inflammatory state and, consequently, coronary microvascular endothelial dysfunction (Paulus, W. et al. 2013). The latter, causes cardiomyocyte hypertrophy and interstitial fibrosis, promoting high diastolic left ventricular stiffness. So far, it is not completely understood the mechanism behind endothelial inflammation and dysfunction in HFpEF. To evaluate the expression of inflammatory and oxidative stress markers, conditioned media (CM) from ZSF1 rats (Lean and Obese), as well as serum obtained from HFpEF patients and healthy volunteers, were exposed to normal endothelial cells (ECs). Both ZSF1-Obese CM and serum from HFpEF patients induced normal ECs to a phenotype of inflammation and oxidative stress, characterized by E-selectin and VCAM-1 upregulation, and eNOS downregulation in the first 10 hours of exposure. Moreover, only ZSF1-Obese rats and HFpEF patients showed an overexpression of the same adhesion molecules at the heart, both at the endothelium and other regions of the heart tissue, indicating a general inflammation response. We also investigated cell senescence in HFpEF, given its strongly relation with inflammation. Preliminary results indicated a link between inflammation and cell senescence, which might lead to a new paradigm for the treatment of HFpEF.

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failure, from comorbidities to personalized medicine"; and Fundação para a Ciência e Tecnologia project: "An iPSC-derived vascular model of Progeria to identify mediators for smooth muscle loss" (Ref: 029229).

P170-T | Pathogen-associated molecular patterns as culprits for mitochondria-driven Parkinson disease neurodegeneration

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Parkinson's disease (PD) is caused by the degeneration of dopaminergic neurons in the substantia nigra pars compacta and is characterized by motor as well as gastrointestinal symptoms. The pathological hallmark of PD is an accumulation of misfolded α-synuclein aggregates within the brain and in the enteric nervous system of earliest and asymptomatic patients. Our central hypothesis argues that gut dysbiosis allows bacterial metabolites to reach the brain triggering neuronal innate immune responses, antimicrobial peptides production, mitochondrial dysfunction, inflammation and ultimately sporadic PD.

Mesencephalic neurons and C57BL/6 mice (6-month-old) were exposed to a gut bacterial metabolite (PAMP-X). Mitochondrial function was assessed in a Seahorse equipment, reactive oxygen species (ROS)using MitoPy dye, mitochondrial network status with TOM20 and cardiolipin exposure using 10-nonyl acridine orange dye. Neuronal innate immunity activation: TLR protein levels, IL-1β, caspase-1 activation and cytokine production. Mice motor function: beam walking and the hindlimb clasping behavior tests.

Our in vitro results showed that PAMP-X targeted the mitochondria decreasing respiration, ATP synthesis, membrane potential and increasing ROS production. Moreover, mitochondrial network was fragmented and trafficking impaired, besides that autophagic turnover was reduced by PAMP-X. We detected higher α-synuclein oligomers levels and neuronal innate immunity activation, with increases in TLR4 and pro-IL-1β levels and in caspase 1-like activity, in the presence of PAMP-X. In vivo studies showed that PAMP-X induced mesencephalon mitochondria dysfunction, decreased mesencephalon TH levels and induced motor dysfunctions.

Altogether, these results may represent new important knowledge on the role of neurotoxins from bacterial origin in the activation of PD brain neurodegenerative process.

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P171-T | Altered neurological fetal prognosis caused by gestational obesity in an animal model

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Background: Obesity represents the most common comorbidity in pregnancy in developed countries, causing numerous obstetrical complications, both on short term and on the long term for the descendants. We aim to show the consequences of maternal obesity on fetal neurological prognosis.

Material and methods: We used an animal model to study the effects of gestational obesity using 30 obese female Wistar rats, in which we induced obesity by high-calorie high-fat diet fed by gavage. The pregnant females were divided in a group receiving normal diet and another one continuing the fat alimentation during gestation.

Results: We analyzed the secretion of adipokines from maternal venous blood (leptin and adiponectin), lipid peroxidation levels (MDA malonyl-dialdehyde) and antioxidation (GSH glutation), maternal and fetal brain homogenates and venous samples. Brain tissue from pups and females was analyzed in hematoxylin eosin stain. Low levels of adiponectin and increased of leptin positively correlated with the high brain lipid peroxidation and low GSH. Lipid peroxidation in brain tissue showed high levels of peroxidation in the group with fat diet, and low levels of antioxidants. Brain histology of obese females continuing fat diet and their pups showed multifocal cerebral necrosis of Purkinje neurons, perineuronal edema with non-specific vacuolization of the molecular layer. The gray matter in the focal cortex was marked by neuronal necrosis with increased basophilia of the cytoplasm accompanied by perineuronal edema and glial cells infiltration.

The clinical observation of the pups that lived showed a lower reactivity and motor activity in the fat group.

Conclusions: Our experiment confirmed histopathological alteration of the brain tissue (especially cerebellum) in foetuses derived from obese rats, correlated with metabolic syndrome diagnosed through peroxidation and adipokine shift and animal behavior. We suggest that the long term neurological prognosis of these foetuses is inferior to the descendant of normal weight mothers.

P172-T | Condition of the spinal motor center of the rat soleus muscle during hypogravity and hypogravity combined with spinal cord stimulation

Artur Fedianin[1]; Nailia Galiullina[1]; Irina Lvova[1]; Tatyana Baltina[1]; Anton Eremeev[1]

[1]*Kazan (Volga region) Federal University, Kazan, Russia*

The study of the central mechanisms of motor function reorganization and the search for new effective methods of motor rehabilitation are important fundamental tasks of modern physiology and medicine. The aim of the work was to assess the functional state of the motor center m. soleus rats with hypogravity and hypogravity combined with electrical stimulation of the spinal cord. The study was carried out on laboratory rats weighing 180-200 g with observance of all bioethical norms in the following experimental series: (a) with hindlimb unloading by the standard method (HU group: n = 7); (b) with hindlimb unloading in combination with daily electrical stimulation of the spinal cord at the level of the L1 segment (HU+ES group: n = 7). After 7 days of exposure to the experimental conditions by electromyographic methods, reflex excitability of the soleus motor neurons was assessed. The control was the data obtained in the study of intact animals (n = 5). It was found that in the HU group, an increase in the reflex excitability of the motor neurons of the motor center of the soleus muscle was observed. In the HU+ES group, no changes were found in the state of the soleus motor center. Thus, with hypogravity, the reflex excitability of the motoroneurons of the soleus muscle increases, stimulation of the spinal cord prevents a change in the state of the motor centers.

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P173-T | Acute pathology of the abdominal organs affects on the reflex excitability of spinal motor neurons

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The development of protective tension of the muscles of the anterior abdominal wall and the lumbar region during inflammation of the appendix is the main diagnostic symptom of the presence of pathology to date. However, influences from the source of nociceptive irritation on skeletal muscle activity can also be inhibitory, reducing the induced activity of the corresponding muscles. The mechanisms of development of such reactions remain unclear and may be associated with changes in the excitability of spinal motor neurons. The purpose of this study was to test the reflex excitability of spinal motor neurons that innervate the m. triceps surae in patients with acute appendicitis. The study involved 20 healthy subjects and 47 patients with acute appendicitis with their informed consent. H and M responses of the medial gastrocnemius and soleus muscle were recorded. It has been shown that in healthy subjects reflex excitability of the right and left spinal motor neurons is almost the same. Nociceptive influences from the inflamed appendix cause a decrease in the reflex excitability of motor neurons of the gastrocnemius and soleus muscles. In the majority of patients, along with general inhibition of the activity of motor neurons, a change in the parameters of H-reflexes was observed, indicating an increase in the reflex excitability of sacral motor neurons on the side of pathology. In 16% of patients, nociceptive afferentation led to increased inhibition of reflex excitability of the sacral motor neurons on the right, despite the typical location of the appendix. The findings suggest a modulating effect of nociceptive visceral irritation on the reflex excitability of spinal motor neurons.

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P174-T | Hyperhomocysteinemia induces whisker pad allodynia and increases the sensitivity to cortical spreading depression in rat cortex

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Background: Hyperhomocysteinemia (HHcy) is a well-known independent risk factor for cardiovascular diseases,

neurodegenerative, metabolic diseases. Population studies demonstrated a possible association between migraine with aura and homocysteine level. Cortical spreading depression (CSD) underlies the phenomenon of migraine with aura. The aim of the present study was to analyses the peripheral mechanical sensitivity and susceptibility to CSD of rats with prenatal HHcy.

Materials and methods: Experiments were performed on Wistar male rats at the age of 6-8 weeks. The rats of the control-group were born from females fed with a control diet. The rats of the HHCy-group were born from females fed with methionine diet. The concentration of homocysteine in control was 6.4 ± 0.9 μmol/L and in HHCy-group 18.1 ± 0.8 μmol/L. Mechanical allodynia was evaluated using the Von Frey filaments (ranging from 0.008 to 2 g of target force, Ugo Basile, Italy) in the whisker pad in ascending order of their strength. CSD and multiple unit activity (MUA) was recorded in somatosensory zone using 16-site linear silicon probes (NeuroNexus Technologies, USA), evoked by the application of 10 μL of KCl solution in increasing concentrations (0.01-1 mol/L) on the visual cortex.

Results: The rats from HHcy-group demonstrated mechanical allodynia in the vibrissae zone and the thresholds of tactile sensitivity were 0.065 ± 0.01 g in the control-group and 0.025 ± 0.005 g in the HHcy-group (pU ≤ 0.01). Moreover, in HHcy rats CSD was evoked by KCl at lower concentration (0.07M) compared to control (0.16M). The frequency of the Background

MUA was significantly higher and a larger increase of MUA frequency at the onset of CSD was observed in HHCy-group.

Conclusion: It was concluded that HHcy induces mechanical allodynia and increased sensitivity to the occurrence of CSD which may underlie the higher risk of migraine attacks in patients with high plasma homocysteine level. This study was supported by Program of Competitive Growth of KFU and RSF №19-15-00174.

P175-T | Cholinergic excitation of trigeminal meningeal nerves as triggers of migraine pain

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Migraine is the most common neurological disorder, which is difficult to treat due to lack of understanding of the neurochemical mechanisms triggering nociceptive signalling. Nociceptive signals leading to migraine headache are generated in the endings of the trigeminal nerve in meninges surrounding brain. Despite the morphological evidence of the parasympathetic innervation of meninges, insufficient attention is currently paid to the role of the cholinergic mechanisms in peripheral nociception. Parasympathetic fibers, innervated the meningeal tissue, release acetylcholine (ACh), which can excite trigeminal nerve fibers initiating nociceptive firing. However, the role of nicotinic receptors in the mechanisms of peripheral trigeminal pain is not understood.

Experiments were performed at the trigemino-vascular system of rats (P 35-45) where was used isolated hemiskull with intact dura mater, dural vessels and nerve, which allows to study molecular mechanisms underlying first steps of a headache.

By using electrophysiological recordings from rat meningeal nerves, we show here that ACh can activate nociceptive signalling in these trigeminal afferents. Moreover, this excitatory action was reproduced by choline known as the agonist of alpha 7 type of nicotinic receptors. Also, we showed cobratoxin (antagonist nicotinic receptors) reduced the number of trigeminal spikes in the control condition. Taken together, our data suggest novel cholinergic mechanisms of trigeminal nociception which can take part in migraine pain.

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P176-T | Intensive antibiotic administration generates motor deficits and neuronal mitochondria impairment

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Parkinson's disease (PD) is characterized by motor dysfunctions, gastrointestinal (GI) symptoms and mental impairment. An imbalance in the gut microbiome is tightly associated with GI complications as well as with a spectrum of neurodegenerative disorders. We hypothesize that a chronic and intensive antibiotic treatment leads to an alteration in gut microbiome and thus potentiates the brain neurodegenerative process, with detrimental effects on motor and neuronal function.

7.5-month-old C57BL/6 male mice were treated with a cocktail of antibiotics in drinking water for 6 weeks. The success of this treatment was determined by mice fecal output

and NGS gut microbiome detection. At this time, a battery of behavior tests was conducted to assess motor and odor functions, as well as cognitive performance. Afterwards, 40-week-old mice were euthanized and brain mitochondria were isolated to measure oxygen consumption rate with the Seahorse apparatus.

Mice treated with the antibiotic cocktail presented a higher fecal output and an altered gut microbiome compatible with gut dysbiosis. Indeed, we observed that mice appendix after antibiotic treatment was 3 times enlarged as compared to controls. Antibiotics administration induced loss of odor discrimination, alteration in motor function and gastrointestinal dysfunction. Finally, antibiotic treatment in vitro and in vivo induced mesencephalic mitochondria dysfunction.

Our results demonstrate that chronic antibiotic administration can have deleterious effects, probably through gut dysbiosis, since antibiotic-treated mice developed early symptoms of neurodegeneration.

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P177-T | The effect of aging on auditory function

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Background: The study aims to support clinical rehabilitative audiology with a quantitative, model-based and statistically anchored system of analysis and prediction methods for assessing the effect of age and individual hearing loss of speech.

Materials and methods: 401 listeners from 19 to 87 years were examined in two clinical centers: Department of Medical Physics and Acoustics, University of Oldenburg, Germany (N = 284) and the State Medical University of St. Petersburg, Russia (N = 117) using the pure tone audiometry and speech audiometry in noise (in Oldenburg - Goettingen Sentence Test, GOESA; in St. Petersburg - Russian matrix sentence test, RUMatrix). Listeners were divided into four groups: young and middle-aged (<60 years) normal-hearing (PTA ≤25) {yNH} and hearing-impaired (PTA >25) {yHI}; older aged (≥60 years old) normal-hearing {oNH} and hearing-impaired {oHI}. Pure tone averaged across frequencies 0.5, 1, 2, and 4 kHz (PTA) and speech reception threshold in noise (SRT_N) were compared. The analysis was performed separately on German and Russian cohorts and additionally cross-validated on mixed data.

Results: Significant differences could be observed between HI and NH in PTA ($P < 0.05$) and in SRT_N ($P < 0.001$) in both cases of old and young listeners (including Bonferroni correction). No significant differences were found between SRT_N in yHI and in oHI ($P < 0.05$). Next, SRT_N in yNH differed significantly from oNH listeners ($P < 0.05$) as well as from yHI and oHI listeners ($P < 0.002$). Also oNH differed from both HI groups ($P < 0.001$). These results were confirmed by data from both centers independently.

Conclusion: Our results indicate that the combination of age and PTA level might be used as a potential predictor of SRT_N. Additionally the GOESA and RUMatrix tests were in both qualitative and quantitative agreement allowing to merge two datasets for German and Russian speakers for further analysis.

P178-T | Adverse drug reactions with fatal outcome in the elderly: a 5-year review of spontaneous reports to the Portuguese pharmacovigilance system

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Background: Adverse drug reactions (ADRs) represent an public health concern, being responsible for considerable morbidity and mortality in older people. The aim of this study was to analyze the suspected ADRs in elderly spontaneously reported to the Portuguese Pharmacovigilance System (PPS) over a 5-year period.

Material and methods: A retrospective analysis of suspected ADR reports involving patients aged 65 years or older received by the PPS between 1 January 2013 and 31 December 2017 was carried out. The cases were assessed according to their seriousness. The medicines involved were categorized according to the WHO Anatomical Therapeutic

Chemical classification system. The terminology used to code suspected ADRs was based on medical terms coded according to the Systems Organ Classes.

Results: After data cleaning, including duplicate identification and assessment of reports without age indication and considering only the spontaneous cases reported, 3692 cases were found. Among the 2458 serious cases of suspected ADRs, 835 (34.0%) led to hospitalization and in 143 (5.8%) of them occurred a fatal outcome. The suspected ADRs most associated with fatal outcome belong to general disorders and administration site conditions (18.5%) and infections and infestations (11.6%). Antineoplastic agents and antithrombotic agents were the most represented pharmacotherapeutic groups of suspected drugs involved (25.0% and 13.6% respectively). In the group of the antineoplastic agents, the other antineoplastic agents (43.6%) and the alkylating agents (23.6%) were the pharmacological subgroups most represented. The antithrombotic agents were represented by the heparin group (36.7%) and the direct factor Xa inhibitors (36.7%). Most of suspected ADRs were observed in males (51.0%) belonging to the age group of 65 to 74 years (52.4%).

Conclusions: Pharmacovigilance databases are important tools to evaluate issues related to the safety of drugs in older people, enabling to improve the knowledge on the safety profile of medicines in these patients.

P179-T | The increasing burden of aging on admission, hospital stay and diagnosis-related group reimbursement. An ordinary day in an Academic Division of Internal Medicine in Southern Italy

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Background: The Italian population is aging; in 2015 (ISTAT) subjects older than 65 years were 21.7% and figures are growing. This trend has enormous impact on national health systems.

Aims: To describe the impact of aging on the hospitalization profile and diagnosis-related group (DRG) reimbursement in an academic Division of Internal Medicine, in Southern Italy.

Material and methods: Data obtained from hospital registry of planned and emergency admissions of patients ranging 15-100 years on two consecutive years (January 2016 to December 2017) were included.

Results: 1820 patients were admitted (899 F, 921 M, mean age 69.0 ± SD16.6 years); 1244 patients (68.3%) aged ≥ 65 years. Patients from Emergency Room (ER) were 72.9% of total admissions, were older than elective patients (mean age 71 ± 16 vs 64 ± 17 years, $P = 0.0000$), had longer hospital stay (8.9 ± 7.7 vs 4.4 ± 4.6 days, $P = 0.0000$) and higher DRG reimbursement (€2899 ± 1660 vs 2291 ± 1789, $P = 0.0000$). Hospital stay ($r = 0.14$; $P = 0.0000$) and DRG reimbursement ($r = 0.19$; $P = 0.0000$) increased significantly with age.

The impact of aging on hospital stay and DRG reimbursement was further evaluated on four age groups, i.e., <65, 65-74, 75-84 and ≥ 85 years (31.7%, 23.6%, 28.7% and 16.0% of total, respectively). A progressive increase at each age group was observed for mean DRG reimbursement (€2340 ± 1937, 2837 ± 1721, 2909 ± 1479, 3045 ± 1501 respectively, $P = 0.0000$) and mean hospital stay (6.6 ± SD7.4, 7.6 ± SD6.8, 8.3 ± SD7.5, 8.9 ± SD6.8 days respectively, $P = 0.0000$).

Conclusions: In an academic division of Internal Medicine in Southern Italy opened to ER, geriatric patients account for 68.3% of total admissions. This population drains major clinical and economic efforts. Geriatric expertise and cost-effective strategies are urgently required to cope with elderly and fragile patients' management.

P180-T | Cytokines levels in rats with prenatal hyperhomocysteinemia

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Background: Hyperhomocysteinemia (hHCY) characterized by an elevated level of homocysteine in blood plasma due to nutritional disbalance, mutations of genes of homocysteine metabolism, lifestyle, etc. Population studies revealed that homocysteine levels gradually increased with age and can achieve 20-25 μmol/L in elderly people which corresponds to mild hHCY. High levels of homocysteine are associated with the incidence of degenerative metabolic diseases, including atherosclerosis, cancer, inflammatory and autoimmune diseases, dementia. It became apparent that many affected patients exhibited features of accelerated aging. The aim of our study was to analyze the profile of pro-inflammatory cytokines in serum of rats with hHCY.

Materials and methods: In present study we used adult rats with prenatal hHCY (homocysteine level ~20 μmol/L), born from female rats fed with methionine rich diet during pregnancy. Cytokines profile in samples of blood plasma was

analyzed using Bio-Plex Pro Rat Cytokine 23-plex Assay (Bio-Rad).

Results: In the model of prenatal hHcy the fetus developed under high level of homocysteine and the offspring demonstrated low body weight, high mortality, the delayed neurobehavioral maturation and violations of motor activity, deficit of exploratory and cognitive abilities in early and late postnatal life. We found that in blood plasma of hHCY rats IL-1α, IL-6, IFN-g, TNFα, EPO were up-regulated compared to the control group.

Conclusions: Our results demonstrated that the chronic hHCY conditions increase the level of pro-inflammatory cytokines in blood serum which impact in on the aging process. The induction of TNFα, which influence the lipid metabolism, coagulation, endothelial dysfunction, in turn may increase the production of IL-1α, IL-6, IFN-g. The increased level of EPO seems to be a consequence of hypoxic conditions due to chronic vascular inflammation and chronic renal failure resulted from glomerular damage in hHCY.

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P181-T | Modulation of evoked cortical activity by the sensory input in the somatosensory cortex of the neonatal rat

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Being a part of somatosensory system, barrel cortex in rodents plays an important role in animals' contact with external world. Each cortical column, so-called - barrel, receives sensory input from corresponding vibrissae on the whisker pad of an animal. Sensory feedback from vibrissae allows rodents to discriminate surface of the obstacle. While sensory coding in adult rodents is a subject of intensive research, little is known about the emergence of sensory coding. To answer this question we have done the experiments on 4-7 days old neonatal rats. Multichannel silicon probes were used to register extracellular activity in the barrel cortex. Using the method of intrinsic optical signal recording we detected the barrels corresponding to stimulated vibrissae and placed electrodes into them. Several stimulation protocols with different interstimulus intervals and speeds of vibrissa deflection were used to characterize properties of the evoked cortical response. Our results demonstrated modulation of evoked cortical response in barrel cortex by different types of stimulation. While speeds of vibrissae deflection more than 0.005 rad/ms evoked oscillatory response with constant and stable number of spikes per trough, lower speeds of vibrissae deflections were associated with progressive increase of MUA per trough (6.8+-2.3 spikes/trough for the 1st cycle of local field potential deflection to 12.7+-2.9 spikes/trough for the 5th cycle). Changes of interstimulus interval also modulated the evoked cortical response. The decrease of a time period between stimulations of the adjacent vibrissae resulted in the reduction of the gamma frequency component of the evoked cortical response for the second stimulus. Based on our results we suggest that mechanisms of sensory coding emerge early in development coinciding with the critical period of cortical maps formation. The work was supported by RFBR grant No.18-34-00924\18 and by RSF grant 16-15-10174.

P182-T | Effects of anesthesia on the sensory evoked optical intrinsic signal in the somatosensory cortex of the neonatal rat

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Optical intrinsic signal (OIS) is the neuroimaging technique that is widely used for detection of the active cortical region position both in fundamental and clinical studies. In fundamental neuroscience, the OIS recordings require the deep anesthesia to immobilize the animal. While effects of the widely used anesthetics on the OIS in adult brain is a subject of active investigations, little is known about the anesthesia effect on the OIS in the immature brain. In the present study, we attempted to answer this question using the neonatal somatosensory system in vivo and widely used anesthetic agents - urethane and isoflurane. The study was done on neonatal rat pups of two age groups: 6-10 and 13-15 days from birth, which correspond to the early postnatal and juvenile periods of rodents development. The amplitude-temporal parameters of OIS were analyzed. Our results showed a negative correlation between the OIS amplitude and the anesthetics concentration. However, while in the younger group of rodents, the increase of the anesthetics concentration resulted in a progressive decrease in the amplitude of OIS, in older group the concentration dependences had the complex form. While the increase of the urethane up to 1 g/kg weakly affects OIS, its further increase resulted in a rapid drop of the OIS amplitude and in all cases, the animal died at 2 g/kg urethane concentration. Low OIS sensitivity in the older group also was seen to the isoflurane, surprisingly, following isoflurane increase was associated with the slower reduction of the OIS amplitude.

In the present study for the first time, it is demonstrated the age difference of the OIS dependence on the urethane- and isoflurane-based anesthesia. We suggest that the different effects of the anesthesia on the OIS are linked to the immaturity of the neurovascular coupling. The work was supported by RSF grant 16-15-10174.

P183-T | Mechanisms underlying the generation of the intrinsic optical signal in the somatosensory cortex of the neonatal rat in vivo

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The intrinsic optical signal (IOS) is a widely used technique for functional mapping in the central nervous system both in clinical and fundamental studies. While in the adult brain the hemodynamic component of the IOS predominates in its generation, in the developing nervous system IOS largely depends on changes of the light-scattering of the active neuronal tissue. In the adult brain in vitro, it was demonstrated that the tissue component of the IOS is linked to the cell swelling, because of the accumulation of chloride and water in the cell during its activity. However, little is known about the mechanisms underlying the formation of the tissue response of IOS during the early postnatal period. To answer this question, we developed the superfused neocortex preparation in vivo and combined it with the IOS imaging in the somatosensory system of the newborn rats (from 4 to 7 days after birth). The functional role of principal chloride cotransporters (NKCC1 and KCC2) was investigated with the help of their antagonists. We have found that inhibition of the NKCC1 cotransporter by the application of the bumetanide (20 uM) into the perfusion chamber weakly affected the IOS signal. However, application of the furosemide in the concentration that selectively blocks the KCC2 (2 uM), strongly modified the amplitude-temporal characteristics of the IOS (for amplitude from $0.07 \pm 0.01\%$ to $0.04 \pm 0.001\%$, for the decay time from 6.4 ± 0.14 s to 9.4 ± 0.3 s, $P < 0.05$, $n = 11$). Thus we demonstrated that while upload of the cell by chloride played the minor role, the functioning of the chloride extruder - KCC2 strongly affected the IOS generation, we suggest that this developmental phenomenon is linked to the low expression level of the KCC2 at the early postnatal period. The work was supported by RSF grant 16-15-10174.

P184-T | Understanding the cell growth profile of lymphoblasts to study as a model to discover the amyotrophic lateral sclerosis disease mechanisms

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Background: Lymphoblasts are a valuable cellular platform to predict the efficacy of therapeutic approaches in preclinical assays, and a reliable model to investigate metabolic alterations in amyotrophic lateral sclerosis (ALS) disease. We studied the growth profile of lymphoblast from ALS patients and sex/age-matched controls to clarify the influence of SOD1 mutations (mutSOD1) on lymphoblast proliferation.

Methods: Lymphoblasts from 3 patients with mutSOD1 and from 3 patients with undetermined SOD1 mutation (undSOD1), as well as 3 sex/age-matched controls were obtained from Coriell Cell Repository. The number of cells were determined using a BioRAD TC20 automated cell counter, and cell viability was assessed using trypan-blue staining. Data are expressed as mean±SEM and comparisons between groups were performed by Two-Way ANOVA and Tukey post-test, $P < 0.05$ considered significant.

Results: Lymphoblasts from 46 years-old patients with undSOD1 presented higher cell growth rates compared to control lymphoblasts in the female ($P < 0.01$) and male groups ($P < 0.001$). In the 26 years-old male group, control lymphoblasts showed a higher cell growth rate compared to mutSOD1 ($P < 0.001$) and undSOD1 lymphoblasts ($P < 0.001$). In male-derived control lymphoblasts, as well as lymphoblast from male patients with mutSOD1 and undSOD1, when isolated from 26 years-old individuals, showed higher cell growth rates when compared to the 46 years-old group ($P < 0.05$). On the other hand, the 46 years-old female group showed higher cell growth values in control ($P < 0.001$) and undSOD1 lymphoblasts ($P < 0.001$), relatively to the male group with similar age.

Conclusions: Our results show higher cell growth rates on lymphoblasts from undSOD1 patients with 46 years in comparison to the controls. Lymphoblasts show a sex and age dependent cell growth profile, with higher cell growth rates in the younger age group and with a higher rate of growth in women compared to men.

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Silva is recipient of FCT Post-Doctoral Fellowship (SFRH/BPD/122648/2016).

P185-T | Development of neurovascular coupling in the somatosensory cortex of the neonatal rat

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Optical intrinsic signal (OIS) is a neuroimaging technique that is widely used for cortical activity mapping in vivo. Previously we have demonstrated that in contrast to the adult brain, where OIS is predominantly based on the hemodynamic changes in the active cortical spot, the changes in light scattering of the active neuronal tissue play the key role in the generation of the OIS in neonates. The mechanisms of the light scattering predominance in the OIS generation is thought to be linked to the immaturity of the neurovascular coupling. To assess the question of the neurovascular coupling maturation we used the model of somatosensory cortex of the newborn rat in vivo and characterized the hemodynamic and light scattering changes of the active and surrounding neuronal tissue during the first two postnatal weeks in the neonatal rat pups. To estimate the level of the neurovascular immaturity we measured OIS signal in the barrel cortex and in the other cortex regions during sensory activation of all whiskers. We demonstrate a progressive decrease of the time delay between evoked electrical activity and hemodynamic response from 1.5 ± 0.7 sec on P6-7 down to 0.2 ± 0.2 sec on P14-P15. We also found that starting from the second neonatal week time-delayed OIS signal was also observed in non-activated regions surrounding barrel cortex. Moreover, the latency between hemodynamic response in active and non-active areas also showed developmental maturation and decrease from 3.2 ± 3.1 sec on P8-9 down to 3.2 ± 3.1 sec on P14-P15. We suggest that maturation of the neurovascular coupling comprises the rise of the reaction time rapidity as well as the increase of the area of blood flow rate modulation.
This work was supported by RSF grant 16-15-10174.

P186-T | Developmental changes in SPW during the early postnatal period

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The early stages of postnatal development are characterized by expression of the specific neuronal activity patterns in the various brain structures. It is widely accepted that these activity patterns are involved in the sculpturing of the highly organized brain. Sharp wave (SPW) is one of these types of activity, that is observed in the developing hippocampal networks. While the mechanisms underlying the sharp waves generation are the subjects of active research, the developmental aspect of the SPW properties remains largely unclear. To characterize the developmental profile and the features of the SPWs the experiments were done on the neonatal rat pups during two first weeks after birth (P4-10, where P0 is a day of birth). The neuronal network activity was recorded in the CA1, CA3 and dentate gyrus regions of the hippocampus in vivo using a multichannel silicon probe. To characterize the developmental profile of the SPWs, their occurrence and current source density profile were analyzed.

We have found the age-related phenomenon with progressive increase of the SPWs rate during the first two postnatal weeks (from 13 ± 6 SPW/min, in P4-7 rats to 33 ± 17 SPW/min in P8-10 rat), that is in agreement with other studies. Nevertheless, the greatest number of the recorded SPWs were seen both in CA1 and CA3 regions and had two sinks of the generation, the local SPWs (0.6% of all SPWs), with single sink in stratum radiatum of CA1 region, were also seen during the first postnatal week. Analysis of their amplitude-temporal characteristics did not show their significant difference with the "double sink" SPWs.

We suggest that the developmental acceleration of the SPWs rate and the transient persistence of the local SPWs are associated with the maturation of the hippocampal network. The work was supported by RFBR grant No.18-34-00924\18.

P187-T | Neurotrauma: expression of signaling proteins in the rat dorsal root ganglia after sciatic nerve transection

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Neurotrauma leads to people disability and death, especially men. However, neuroprotectors capable of protecting neurons are absent. We hope that modulation of signaling pathways that control cell death may be neuroprotective. We studied expression of different signaling proteins belonging to various biochemical subsystems in the rat dorsal root ganglia (DRG) after transection of sciatic nerve (SN) that was used as a neurotrauma model. DRG volume and the number of neurons in these ganglia did not change 1 and 7 days after SN transection. Apoptosis of few DRG neurons was observed only at 7 days after axotomy. Glial cells were more vulnerable. At 24 hours after SN axotomy,

the level of glial apoptosis was threefold higher than that in control contralateral ganglia, and 6-fold higher at 7 days. At 24 hours after SN axotomy, ubiquitin hydrolase UCH-L1, caspase 3, and protein p53 were significantly overexpressed in DRG ganglia as compared with their levels 1-4 hours after nerve transection. However, they did not differ from that in contralateral control ganglia within first 1-24 hours after axotomy. These non-specific changes were possibly associated with the systemic response of the rat nervous system to local nerve injury. The changes in the levels of transcription factor E2F1 and histone deacetylase HDAC1 were more specific. The expression of E2F1 significantly increased relative to control at 4 hours after SN transection. The level of HDAC1 in the axotomized DRG ganglia exceeded the control levels earlier - at 1-4 hours after SN transection. Such early changes indicated their regulatory and promoting roles in DRG responses to SN axotomy. These proteins can serve as potential markers of nerve damage and/or targets for therapeutic intervention. The work was supported by Russian Ministry of Education and Science, grants 6.6324.2017/8.9 and 6.4951.2017/6.7.

P188-T | Clinical trials of DHEA and pregnenolone in depression - a systematic review and meta-analysis

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Background: The interest on the benefits of using the steroids such as dehydroepiandrosterone (DHEA), allopregnanolone and other neurosteroids on various aspects of human mental health, including in the regulation of mood, is increasing. However, it is needed to evaluate if they have a clear benefit in the treatment of depressive symptoms.

Material and methods: This study aimed to review the scientific literature on the use of DHEA and pregnenolone in the treatment of depression and depressive symptoms and perform a meta-analysis. For this, PubMed, ISI Web of Knowledge, Cochrane Library and Scopus databases were searched using the following terms: depression, treatment, DHEA, clinical trials and neurosteroids.

Results: We analysed, for DHEA, the response rates ($Q = 5.91$, $P = 0.12$, $I2 = 49\%$), the DHEA levels ($Q = 0.2$, $P = 0.88$, $I2 = 0\%$) and HAM-D scores ($Q = 1.24$, $P = 0.23$, $I2 = 30\%$). For pregnenolone HAM-D scores ($Q = 1.42$, $P = 0.23$, $I2 = 29\%$) and response rates ($Q = 2.21$, $P = 0.14$, $I2 = 55\%$) were explored.

Conclusions: We concluded that the studies published to date indicate promising results regarding the use of DHEA and pregnenolone in the treatment of depression and depressive symptoms.

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P189-T | Efficacy of non-pharmacologic treatment in adolescents (17-18 years old) with tension-type headache associated with pericranial tenderness with and without alexithymia

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Background: Previously, we established efficacy of elastic kinesio taping (KT) for the treatment of tension-type headaches (TTH) in children. However, we registered adolescents for whom this method was not sufficiently effective. A detailed study revealed the high level of alexithymia in them.

Material and methods: Diagnosis was conducted according to the International Classification of Headache Disorders, 3rd edition (beta-version) and Toronto Alexithymia Scale (TAS-20). We examined 103 adolescents with frequent episodic and chronic TTH with pericranial tenderness, who kept a headache diary for 30 days prior to the therapy. KT was performed every 5 days for 30 days. The fundamental principle of the method is the modeling of the muscular-fascial segment.

Results: The average age of the patients was 17.2 ± 2.2 years. The study included Group-1 (61 patients) with low level of alexithymia (TAS-20 <51 points), Group-2 (42 patients) with high level of alexithymia (>61 points). The average indicators in Group-1 were: headache intensity by the Visual Analogue Scale (VAS) 61.8 ± 1.1 points, days with a headache 18.5 ± 4.1, TAS-20 45.2 ± 2.1 points; in Group-2: headache intensity by VAS 60.2 ± 2.4 points, days with a headache 18.8 ± 4.7, TAS-20 68.2 ± 5.9 points. There was no statistically significant difference between points by VAS and days with headache in two groups. After 30 days we compared the figures in the groups before and after treatment. The average indicators in Group-1: headache intensity by VAS 42.1 ± 0.8 points ($P < 0.05$), days with a headaches

10.1 ± 1.6 days ($P < 0.05$); in Group-2: headache intensity by VAS 57.8 ± 1.7 ($P > 0.05$) points, days with a headaches 16.5 ± 6.6 days ($P > 0.05$).

Conclusions: KT is effective and safe method to eliminate pericranial tenderness in adolescents with TTH without alexithymia. However, in adolescents with alexithymia KT is not effective, and they need additional treatment.

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P190-T | Features of neurological status in children with speech development disorders (sensorimotor alalia)

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Background: Diagnosis of neurological symptoms in children with sensorimotor alalia (SMA) helps adequately plan and conduct speech habilitation. The purpose of the study is to determine the frequency and variants of neurologic symptoms in children with SMA.

Materials and methods: Neurological examination of 25 ambulatory patients aged 2.2-6.0 with SMA (a disorder of impressive and expressive speech development in the early stages without the previous period of normal speech development according to ICD-10), 18 boys (72%) and 7 girls (22%) with normal conditions for speech development. Exclusion criteria: traumatic brain injury, neuroinfection, dysarthria, rhinolalia, aphasia, hearing impairment, mental retardation, autism, epilepsy, late-talkers. To confirm the diagnosis, all patients underwent neurolinguistic assessment.

Results: 92% of patients had microfocal neurological symptoms. The most common symptoms were: diffuse muscle hypotension in 23/25 (92%), motor skills problems −9/25 (36%), attention deficit −9/25 (36%), pyramidal symptomatology in the lower limbs −7/25 (28%), hyperactivity −7/25 (28%), flat-bottomed stop −7/25 (28%). There was a lack or delay in the ability to jump −5/25 (20%), an atactic gait with a broad support base −4/25 (16%), echolalia −4/25 (16%), unstable convergent strabismus −3/25 (12%), lack of neatness skills −3/25 (12%), non-progressive moderate hydrocephalic syndrome −3/25 (12%), stereotyped movements −3/25 (12%), discoordination −3/25 (12%), hypersalivation, gait with internal rotation of stop socks −2/25 (8%), fatigue −2/25 (8%), nystagmus in the last leads −1/25 (4%), pseudobulbar syndrome elements −1/25 (4%), apraxia elements −1/25 (4%), problems with the chewing −1/25 (4%), asymmetry of the eye slits −1/25 (4%).

Conclusions: The majority of children with SMA had diffuse micro-focal symptoms in the neurological status, frontal-cerebellar symptoms and symptoms of the syndrome of hyperactivity and attention deficit dominated.

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P001-F | Early mitochondrial metabolism and dynamics changes as predictors of neurodegeneration and neuronal cell death

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Background: Neuronal calcium and ATP homeostasis is regulated through alterations in mitochondria fusion/fission events and anterograde and retrograde transport in neurons. Mitochondrial dynamics form an interactome that ultimately controls mitochondrial quality, quantity and metabolism. Our objective is to show that alterations in this interactome lead to point-of-no-return situations characterized as first signs of progressive neurodegeneration.

Material and methods: Differentiated human SH-SY5Y neuroblastoma cells were treated with non-lethal concentrations of either rotenone (31.25, 62.5, 125 and 250 nM) or 6-hydroxydopamine (6.25, 12.5, 25 and 50 µmol/L). Cell viability was determined by measuring cell mass, metabolic activity and [ATP] using SRB, resazurin, and CellTiter-Glo Luminescent Cell Viability assays, respectively, after 96 hours treatment. At earlier time points, before changes on cell viability occurred, cells were labelled with different fluorescent dyes, imaged under a Nikon Ti-E-H-TIRF microscope. Mann-Whitney's test was used for comparison of two-mean values of controls and treated cells.

Results: In a dose-dependent manner, rotenone and 6-hydroxydopamine decreased cell area and number of neuronal processes, leading to the loss of neuronal architecture. Under the same treatment conditions, neuronal mitochondria showed decreased axonal transport (velocities, time and length of movement) along with other dynamic events like fusion/fission cycles that are correlated to mitochondrial shape alterations.

Conclusions: Alterations in mitochondrial metabolism and dynamics lead to neuronal cell morphology modifications and neurodegeneration that can ultimately drive cell death. Characterization and prevention of these changes, prior to the escalation to point-of-no-return situations, may be the answer to diagnosis and to design therapeutic interventions to prevent cell death in neurodegenerative diseases.

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P002-F | Mitochondrial function, biogenesis and dynamics deregulation in ALS patients PBMCs: a potential biomarker to enable a reliable prognosis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of motor neurons. Mostly, ALS is a sporadic disease; just between 10%-15% of the cases is familial. Despite its etiology is multifactorial, the modification in energetic cellular status is one of the main neuropathological features of this debilitating disorder. Because there is no effective therapy for ALS, attention has to be made towards prognostic factors and biomarker that enables a predictable follow-up and therapy efficacy. Thus, the goal of this study was to evaluate the role of mitochondrial function in ALS patients (case-control study). To reach our goal, blood samples were collected from ALS patients and age-matched controls, and peripheral blood mononuclear cells (PBMCs) were cultured with RPMI 1640 medium (with 10% of serum), and maintained overnight at 37°C and 5% CO2. Mitochondrial function was evaluated loading the cells with Fluo-4-AM (10 µmol/L, 1 hours), TMRE (500 nM, 1 hours) and CM-H2DCF-DA (20 µmol/L, 1 hours), to investigate calcium homeostasis, mitochondrial membrane potential and oxidative stress, respectively. Moreover, mRNA was extracted and converted into cDNA in order to study mitochondrial biogenesis and dynamics thought qPCR; it was used primers for PGC-1-alpha, NRF1, NFE2L2, TFAM, DNM1L, FIS-1 and ACTIN (housekeeping). Our data show that mitochondria from ALS patients are depolarized and have a significant decrease in the calcium uptake capacity. Furthermore, ALS PBMCs have a significant augmentation in oxidative stress accompanied by a decrease in NF2EL2 expression, in addition to a significant diminishment in PGC1α, TFAM and NRF-1 expression and an increase in mitochondrial fission. Therefore, we believe that this study could disclose the role of mitochondrial dysfunction as a biomarker to ALS prognosis and therapeutic evaluation.

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P003-F | Hypoxia-induced mitochondrial metabolism deregulation associated to schizophrenia

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In recent years, several studies have shown the relationship of hypoxia with psychiatric illnesses such as schizophrenia (SZ). In this sense, it is believed that the hypertension during pregnancy imposes on the fetus a continuous intrauterine hypoxia regime due to placental vasoconstriction, favoring cerebral vulnerability during development leading, therefore, to SZ. Considering that numerous neural mechanisms, including cellular proliferation, migration and differentiation, in addition to changes in synapsis formation depends on energetic supply (ATP synthesis), the maintenance of mitochondrial metabolism is essential to keep cellular balance and survival. Therefore, in the present work, we evaluated functional parameters related to mitochondrial function in astrocytes from control (Wistar) and Spontaneously Hypertensive Rats (SHR) animals exposed both to chemical and gaseous hypoxia. For this purpose, we used: Fluo-4-AM (10 µmol/L) to measure calcium levels, TMRE (500 nM) to analyze mitochondrial membrane potential and H_2DCF-DA (20 µmol/L) and Mitosox (5 µmol/L) to investigate redox homeostasis. Moreover, real-time PCR and Western blot were used to verify the expression and proteins levels related to mitochondrial metabolism. Furthermore, we also measured high-energy compounds and oxygen consumption. We show that astrocytes after hypoxia presented depolarized mitochondria, disturbances in Ca^{2+} handling, destabilization in redox system and alterations in ATP, ADP, Pyruvate and Lactate levels, in addition to modification in NAD+/NADH ratio, and Nfe2 l2 and Nrf1 expression. Interestingly, intrauterine hypoxia also increase expression of genes and proteins related to mitochondrial content, respectively; Pgc1a, Tfam,

MtCo1 and TOM-40 levels. Altogether, our data suggest that hypoxia can induce mitochondrial deregulation and decrease energy metabolism in the most prevalent cell type in the brain, astrocytes.

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P004-F | Design and synthesis of mitochondriotropic antioxidants based on dietary scaffolds endowed with neuroprotective activity and BBB permeability

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Background: The use of exogenous antioxidants can be a valuable strategy for decreasing oxidative stress status in a wide range of pathological conditions, such as neurodegenerative diseases. Hydroxycinnamic acids (HCAs) are dietary phenolic antioxidants that have been used as promising scaffolds for the development of new antioxidants for diverse applications. However, their hydrophilic character compromises the therapeutic success due to a restrict distribution throughout the targeted sites, in particular mitochondria. Thus, targeting oxidative stress and mitochondrial dysfunction is considered an effective therapeutic approach for neurodegenerative diseases. Consequently, new mitochondriotropic antioxidants (AntiOxCINs derivatives) based on natural HCAs scaffolds have been developed.

Material and methods: We studied the antioxidant and redox properties, iron chelation capability and cytotoxicity in human neuroblastoma cells (SH-SY5Y) of the new mitochondriotropic agents. Additionally, we also studied their cellular neuroprotective profile against diverse oxidative stress-induced damage, as well as, the capacity to cross the BBB using hCMEC/D3 as in vitro brain blood barrier (BBB) model. Statistical significance of treated vs control group was made by using a one-way ANOVA.

Results: The new mitochondriotropic antioxidants showed remarkable antioxidant ($IC50 = 20.7 \pm 1.1$ μmol/L) and chelating properties ($88.5 \pm 0.9\%$), presenting low cytotoxic effects on human differentiated neuronal (SH-SY5Y) cells ($4.69 \pm 1.58\%$) and exhibited significant neuroprotective properties on SH-SY5Y cells against 6-hydroxydopamine (6-OHDA) ($^{**}P < 0.01$) and hydrogen peroxide (H2O2) ($^{****}P < 0.0001$) oxidative insults. Moreover, the new mitochondriotropic compounds were able to permeate (27.7%) a layer of hCMEC/D3 cells in a time-dependent manner.

Conclusions: The new mitochondriotropic agents can operate by minimizing/preventing neurodegeneration through a multi-target mechanism, namely tackling mitochondrial dysfunction, oxidative stress promoted by different reactive species and preventing free iron overload.

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P005-F | Role of estrogen on metabolic remodeling during osteoclast differentiation

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Background: Bone remodeling is a dynamic process involving old bone resorption by osteoclasts and new bone matrix formation by osteoblasts. With aging, changes occur in the organism, influencing bone turnover. Mitochondria, the power house of the cell, also controls cellular differentiation, intermediate metabolism, intracellular calcium balance and the production of Reactive Oxygen Species (ROS). There are evidences correlating osteoclasts differentiation and increased ROS production mediated by RANKL action. Therefore, it is believed that an association between RANKL-mediated osteoclast differentiation, estrogen/estrogen receptors pathway and alterations in mitochondrial activity exists. The two main goals of this study were: 1) differentiate RAW 264.7 macrophages into mature osteoclasts after exposure to RANKL, 2) evaluate how acute exposure to RANKL and estradiol (E2) interfere with mitochondrial respiration.

Methods: Murine macrophages RAW 264.7 where differentiated into osteoclasts upon addition of 50 ng/mL RANKL to the culture medium, up to 6 days. To confirm osteoclast differentiation TRAP staining was performed and Western Blot were used to confirm the presence of cathepsin K. Mitochondrial activity was evaluated by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse XFe96 Extracellular Flux Analyzer. Comparison between obtained data was performed by Kruskal-Wallis non-parametric test.

Results: Our results show that RAW 264.7 cells differentiate into osteoclasts upon exposure to RANKL dying positive for TRAP and expressing cathepsin K. A slight increase (about 14-20%) in basal respiration, OCR associated with proton flux across ATP synthase and OCR associated with proton leak was observed as a result of acute exposure to 100 nmol/L E2 and 1 μmol/L E2.

Conclusion: Our results suggested that E2 appear to interfere with mitochondrial inner membrane permeability which may condition osteoclasts differentiation.

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P006-F | How estrogen modulates mitochondrial function during osteoblast differentiation?

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Background: Osteoblasts, the cells responsible for bone formation, are crucial to maintain bone homeostasis. An unbalance in bone homeostasis can lead to osteoporosis. Estrogen withdrawal during menopause increases the risk of osteoporosis. Mitochondria are important organelles that play an essential role during cell differentiation. The aim of this study was to evaluate the effect of estrogen on mitochondrial performance during osteoblast differentiation.

Methods: Using the MC3T3-E1 cell line, we induced cell differentiation into osteoblasts by adding 50 μg/mL of ascorbic acid and 10 mmol/L of β-glycerophosphate to the culture medium. To study the role of estrogen receptors (ER) on the process, the antagonists MPP (ERα) and PHTPP (ERβ) were added to culture medium. Mineralization was assessed using the Alizarin Red S Staining Assay after 2, 7, 14 and 21 days of differentiation. Mitochondrial performance in the presence of 17β-estradiol (E) for 1, 24 and 48 hours was evaluated by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse XFe96 Extracellular Flux Analyzer. Statistical analysis was performed by Kruskal-Wallis non-parametric test.

Results: Our results show a concentration-dependent decrease in mineralization (about 50%) when MC3T3-E1 cells were differentiated in presence of ER antagonists. Alteration in mitochondrial respiration was observed when MC3T3-E1 cells were exposed to differentiation medium in absence of E, showing a time dependent decrease in basal (16 and 25%), maximal (53 and 36%) and ATP-linked OCR (16 and 43%). After 48 hours of treatment, 10 nM of E increased basal and maximal (by 30%) and ATP-linked OCR (by 40%) in MC3T3-E1 cells.

Conclusions: These results suggest that estrogen deficiency affects osteoblast activity and that estrogen receptor alpha may be responsible for the effects of estrogen on osteoblasts.

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P007-F | Mitochondria performance during rankl-induced osteoclast differentiation

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Introduction: Advanced age and sex steroids deficiency are closely linked to loss of bone mass due to an increase in osteoclast (OC) number and activity. Mitochondria may play a role in this process. Several studies indicate that RANKL stimulates ROS production, critical for OC generation. When compared with their precursors, OCs have lower levels of intracellular ATP associated with increased oxidative stress. The main purpose of this study was to determine how RANKL affects mitochondrial function and dynamics during RANKL-induced OC differentiation.

Materials and methods: Mouse macrophage RAW 264.7 cells were differentiated into OCs through the addition of 50 ng/mL RANK. Cell viability was evaluated by resazurin assay. ROS/oxidative stress were assessed by H2DCFDA, Amplex Red and MitoSox. ATP levels measured by CellTiter-Glo® Luminescent Cell Viability Assay. Cellular metabolic profiling was assessed by measuring oxygen consumption (OCR) and extracellular acidification (ECAR) rates using the Seahorse XFe96 Extracellular Flux Analyzer. Data shown are means±SEM. Kruskal Wallis non-parametric test was performed.

Results: For 6 days of RANKL exposure, our results showed a decrease of 60% in basal and maximal OCR and a decrease of 50% in ATP synthesis-associated OCR. A 50% decrease in extracellular acidification (ECAR) was also observed in the same conditions, suggesting a reduction in cellular metabolic activity. ATP levels decreased by 50% after 6 days exposure to RANKL. The levels of hydrogen peroxide and superoxide anion also increased 50% at that time point.

Conclusions: Our results showed a metabolic remodeling during OC differentiation, suggesting that cellular metabolism could be used as a potential target to manipulate OC differentiation.

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P008-F | Inhibition of CSE decreases H^+-leak in myocardial mitochondria induced by ischemia-reperfusion

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CSE is an enzyme synthesizing a gas transmitter hydrogen sulfide, whose role in the cardiovascular system is still debatable. Previously, we showed that the combination of DL-propargylglycine (PAG) and L-cysteine (Lc, precursor of glutathione synthesis) caused a potent cardioprotective effect, which manifested in the complete restoration of the heart function after ischemia-reperfusion. The aim of this work was to study the effect of PAG+Lc at mitochondrial function in ischemia-reperfusion (IR) model in rats.

CSE was inhibited by DL-propargylglycine (11.3 mg/kg, intraperitoneally, 10 min) followed by L-cysteine administration (120 mg/kg, intraperitoneally, 30 min, PAG+Lc+IR). Ischemia-reperfusion (20 min-10 min) of isolated heart was performed at Langendorff apparatus, then, cardiac mitochondria were isolated and tested for respiration rate, mitochondrial membrane potential ($\Delta\psi$) and with $TPMP^+$-selective electrode and calculated by the Nernst equation. H^+-leak $_m$ was titrated with malonate addition (up to 0.5 mmol/L).

$\Delta\psi_m$ was slightly decreased in IR group (154.3 ± 2.5 vs 157.1 ± 4.0 mV in control), the respiration rate increased significantly (205.5 ± 12.3 vs 165.4 ± 5.3 $nmolO_2$/min/mg protein in control, $P < 0.05$), indicating reduced efficacy of the oxygen utilization by cardiac mitochondria of IR group. PAG+Lc pretreatment increased $\Delta\psi_m$ at reperfusion to 176.7 ± 2.7 mV (vs 154.3 ± 2.5 in IR, $P < 0.001$). An increase in the respiration rate was observed (278.4 ± 11.1 $nmolO_2$/min/mg protein vs in 205.5 ± 12.3 IR, $P < 0.001$). At the same time, shift of the H^+-leak curve to the right, closer to the control curve, indicated increased efficacy of the respiratory chain compared with IR group. The value of $\Delta\psi_m$ in PAG+Lc+IR group was 159.8 ± 1.0 mV at the respiration rate of 115 $nmolO_2$/min/mg protein ($P < 0.05$), then the value in IR group was 150.8 ± 2.6 mV.

Thus, PAG+L-cysteine pretreatment decreased IR-induced H^+-leak, increased mitochondrial membrane potential and improve respiratory chain efficacy upon reperfusion. We suggest that this mechanism underlay the cardioprotection induced by PAG+L-cysteine pretreatment.

P009-F | ANT2 silencing promotes a metabolic shift in P19 embryonal carcinoma stem cells

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Background: Cancer stem cells (CSCs) are characterized by potent self-renewal and survival properties. Cancer cells have altered metabolism, often demonstrating an increased glycolytic phenotype. The inner mitochondrial membrane adenine nucleotide translocase 2 (ANT2) is up-regulated in several cancers and associated with cancer metabolism. Thus, we hypothesized that ANT2 expression may induce a metabolic shift in CSCs.

Material and methods: Mouse P19 embryonal carcinoma stem cells (P19SCs) were used and differentiated by using retinoic acid (RA,1 μmol/L) producing a mixture of cells with mesoderm and endoderm properties (P19dCs). Differentiation and pluripotency markers, and ANT2 levels were evaluated in P19SCs and P19dCs by Western Blot. P19SCs transfected with Control or ANT2-siRNA were evaluated after 48/96 hours for different parameters regarding mitochondria remodeling and cellular metabolism. Western blot and cell viability assay (resazurin assay) were used. Statistical comparisons were carried out using Student's *t*-test. Differences with $P < 0.05$ were considered statistically significant.

Results: Upon treatment with RA, TROMA-I levels increased and OCT4 levels decreased ($P < 0.001$) in P19dCs. Furthermore, ANT2 was highly expressed in P19SCs when compared to P19dCs ($P < 0.05$). In P19SCs transfected with ANT2-siRNA, ANT2 expression was diminished ($P < 0.05$) after 96 hours, which was accompanied by a decrease of 20% in metabolic activity and by an increase of Mitofusin-1 protein levels ($P < 0.05$). Moreover, Hexokinase II protein

levels were decreased after 48 hours of ANT2 silencing ($P < 0.001$).

Conclusions: Our findings demonstrate that ANT2 is more expressed in P19SCs. Additionally, our results also suggest that ANT2 silencing promotes a metabolic adaptation in CSCs towards a less glycolytic phenotype, as well as increasing mitochondrial fusion.

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P010-F | Physical exercise during pregnancy counteracts the deleterious effects imposed by gestational diabetes mellitus on the offspring liver mitochondrial function

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Background: The development of gestational diabetes mellitus (GDM) during pregnancy is associated with impaired glucose tolerance and insulin resistance, which may be transgenerationally inherited by the offspring (F1). The aim of our study was to assess the effects of maternal physical exercise (PE) during pregnancy on F1 liver mitochondrial function in a model of GDM.

Methods: Female Sprague-Dawley fed with control (C) or high-fat-high-sugar (HFHS) diets were submitted to PE during the 3 weeks of pregnancy. Oral glucose tolerance tests (OGTT) were performed before and during pregnancy to assess the GMD condition and in F1 at 6th weeks of age. F1 body weight (BW) was weekly monitored and liver mitochondrial function was determined at 6th weeks of age using complex I and II-related substrates.

Results: No pre-mating OGTT differences were observed between C and HFHS groups. In contrast, increased impaired glucose tolerance was detected during pregnancy in HFHS groups, regardless of PE. Moreover, no differences in glucose tolerance were found in F1. Although F1 of HFHS mothers had significantly higher BW compared to C, PE during pregnancy decreased this adverse effect of HFHS. Regarding F1 liver mitochondrial function, no alterations were found in state 3 and 4 between groups, using complex I and II-related substrates, while a decrease in the respiratory control ratio (RCR) of the HFHS animals was observed using substrates for complex I compared to C. PE was able to improve the F1-related RCR in the HFHS animals back to C values.

Conclusion: HFHS during pregnancy induced GDM despite no effects on F1 glucose tolerance early in life. However, this fed condition during pregnancy had a negative impact on F1 BW as well as in the liver mitochondrial function, which were significantly attenuate by PE.

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P011-F | P-cadherin effect in mitochondrial dynamics and biogenesis of breast cancer cells

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Background: P-cadherin, a cell adhesion glycoprotein, was previously found overexpressed in 30% of breast cancer cases and correlated with poor prognostic and overall survival. P-cadherin influences cell to cell adhesion, migration and invasion in breast cancer cells, as well as it is associated with glycolytic markers, such as GLUT1, CAIX, MCT1 and CD147 in human breast tumor tissue. Since metabolic reprogramming is a hallmark of cancer, mitochondrial alterations may justify this switch. Therefore, our objective was to address P-cadherin role on mitochondrial remodeling in breast cancer cells.

Material and methods: Two breast cancer cells models were used: the MCF-7/Az luminal breast cancer cell model, where P-cadherin was constitutively overexpressed (MCF-7/Az.mock vs MCF-7/Az.P-cad), the second one, where BT20 cells are transfected with control or CDH3-siRNA were used. Expression of proteins related with mitochondrial oxidative phosphorylation (OXPHOS), dynamics and biogenesis were evaluated by Western Blot.

Mitochondrial morphology, network and membrane potential ($\Delta\Psi m$) were evaluated by fluorescence microscopy using TMRM. Mann-Whitney test was used to determine statistical analysis.

Results: MCF-7/Az.P-cad cells showed a 40% decrease in MFN-1 and TOM20 proteins, while OXPHOS and mTFA protein expression were unaltered relatively to MCF7/AZ/Mock. No differences were observed on OXPHOS subunits expression in the BT20 cell model with silenced P-cadherin.

Conclusions: The results suggest that modulation in P-cadherin expression levels may exert some modulatory effect on cells mitochondrial biology with potential impact on breast cancer cell metabolic switch.

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P012-F | Comparative analysis of drugs of abuse toxicity in differentiated SH-SY5Y cells

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Background: Mitochondrial toxicity is common to most known drugs of abuse and can be a hallmark to flag neurotoxic compounds. Our objective is to identify parameters that predict cell toxicity before the onset of cell viability loss.

Material and methods: We exposed differentiated SH-SY5Y cells to standard drugs of abuse: Methamphetamine (Meth: 0.09-10 mmol/L), Cocaine (Coc: 0.625-10 mmol/L), Methadone (Methad: 0.02-5 mmol/L) and Morphine (Mor: 0.3125-5 mmol/L) for 24-72 hours. We analyzed dose- and time-dependent effects on cell viability by assessing ATP content (CellTiter-Glo® Luminescent Cell Viability Assay-luminometry) after 24 hours, metabolic activity (resazurin reduction-fluorimetry) and cell mass (Sulforhodamine B assay-colorimetry) after 48-72 hours. Data was normalized as the percentage of respective untreated controls and statistical significance was set at $P < 0.05$.

Results: We observed a clear decrease in cell viability for increasing concentrations of all drugs of abuse. Coc induced a significant decrease in cell mass starting at 48 hours and 5 mmol/L, with all parameters being decreased at 10 mmol/L. At 2.5 mmol/L, Meth significantly decreased metabolic activity after 48 hours and decreased all parameters starting at 5 mmol/L. Mor induced a significant decrease in ATP levels starting at 2.5 mmol/L. Methad was the most toxic drug, decreasing cell viability at 0.156 mmol/L, whereas at 0.3125 mmol/L all parameters measured were significantly decreased.

Conclusions: Standard drugs of abuse tested presented dissimilar toxicity patterns that may depend on different chemical structures and/or mechanisms of action. Understanding the timeline of events associated with drug toxicity may help to establish a screening protocol for new drugs, predicting their toxicity and flagging them before they reach the consumer.

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P013-F | Pharmacological modulation of Hsp70 alters huntingtin proteostasis in cellular models of Huntington's disease

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Background: Polyglutamine expansions in the huntingtin protein (Htt) cause Huntington's disease (HD), whose pathogenesis is associated with abnormal proteostasis and mitochondrial dysfunction. Inhibition of the ATPase activity of heat shock protein 70 (Hsp70), a main effector of the proteostasis network, with YM-1 was protective in Spinal-Bulbar Muscular Atrophy models. As the modulation of specific Hsps may exert different effects depending on the client protein, we investigated if YM-1 could modulate Htt proteostasis and mitochondrial function in HD cellular models.

Methods: PC12 cells with inducible full-length Htt(Q23/Q145); U2OS cells transfected with N-terminal GFP-Htt(Q23/Q74). Htt and Hsp levels were determined by Western blot. Hsp70-Htt interaction was assessed by co-immunoprecipitation. Htt aggregation was monitored by fluorescence microscopy and filter-trap assay. Cell death was determined according with morphological changes through live-imaging.

Results: In PC12 cells, Hsp70 interacted with mutant Htt (mHtt) but not wild-type Htt (wtHtt), suggesting specific recognition of the abnormal protein. Heat shock (HS) or YM-1 treatment alone altered neither wtHtt nor mHtt levels. Combining HS with YM-1, however, decreased mHtt levels. Since HS increased the expression of Hsp70 and

its co-chaperone Hsp40, these results are consistent with an Hsp70-dependent effect of YM-1. Moreover, YM-1 increased mtHsp70 levels in mHtt-expressing cells, suggesting an activation of the mitochondrial unfolded protein response. In U2OS cells, Hsp70 co-localized with N-terminal mHtt aggregates. YM-1 decreased soluble mHtt levels without altering mHtt aggregation or cell death.

Conclusions: This study supports YM-1 as a therapeutic strategy to limit mHtt accumulation through increased degradation and provide new insights on potential off-target effects of YM-1 on mitochondria.

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P014-F | Modulation of complex I and oxidative capacity in cells under metabolic stress

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Metformin is a biguanide drug frequently used for the treatment of Type 2 Diabetes Mellitus (T2DM), being known to inhibit hepatic gluconeogenesis and decreasing glucose production in liver. Nevertheless, metformin was shown to target small non-coding RNAs - microRNAs (miRNAs) - involved in T2DM and cancer. Recently, miR-378a-3p was found to be regulated by metformin in hepatocellular carcinoma cells. This miRNA is embedded in the PGC-1β gene and was identified as a potential agent on the prevention and treatment of obesity by activating the pyruvate-phosphoenolpyruvate (PEP) futile cycle in skeletal muscle through direct interaction with the AKT1/FOXO1/PEPCK axis. Thus, stimulating the pyruvate-PEP futile cycle in skeletal muscle by activating miR-378a-3p could prove to be an attractive strategy to prevent the accumulation of reducing equivalents at the respiratory chain typically observed in metabolic diseases.

In order to mimic hyperglycaemia observed on the skeletal muscle in metabolic diseases, C2C12 myoblasts and myotubes were incubated with high glucose (25 mmol/L) media. Following miRNA extraction, miR-378a-3p expression levels were analysed by RT-qPCR using specific TaqMan probes. C2C12 myoblasts were successfully transfected with mirVana miR-378a-3p mimics and inhibitors using the Neon Transfection System. Lastly, mitochondrial function and oxidative capacity was evaluated using the Seahorse XF Cell Mito Stress Test kit.

Preliminary data have shown that miR-378a-3p is differentially expressed in C2C12 myoblasts and C2C12 myotubes, with the latter ones presenting the higher expression levels. Furthermore, metformin was shown to elevate miR-378a-3p levels in C2C12 myoblasts previously subjected to hyperglycaemic conditions. They were further implied in the restoration of the oxidative capacity on those hyperglycaemic cells. These results suggest a new mechanism of action through which metformin can act to prevent conditions that are on the basis of metabolic diseases.

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P015-F | Action of lysine (K)-deacetylase modulators on cell metabolism in astrocytes exposed to oxidative stress and excitotoxicity: possible neuroprotective role in amyotrophic lateral sclerosis

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Background: Amyotrophic Lateral Sclerosis (ALS) is a rare disease with no confirmed pathological mechanism. Yet, many are the mechanisms proposed to subsidize the process of neurodegeneration, among them oxidative stress, excitotoxicity and mitochondrial dysfunction. Therefore, the objective of this project was to evaluate the neuroprotective effect of modulators of lysine deacetylases (KDACs) on mitochondrial metabolism and cell survival in primary astrocyte culture after increased oxidative stress (H2O2) and induction of excitotoxicity (after exposure to the neurotoxin LBMAA)

Methods: We used primary culture of C57BL mice. For viability assays, we used MTT test; all cells were treated with H2O2 or LBMAA and four different KDACS (TSA, SB, MS-275 and SBHA). The levels of histone H3 and acetylated histone H3 were evaluated by western blot, mitochondrial membrane potential was investigated by TMRE fluorescent assay. Mitochondrial metabolism was also analyzed by the ATP/ADP, pyruvate/lactate and NAD+/NADH ratio, as well as measurement of oxygen uptake and mitochondrial biogenesis. The statistical analysis was done by One-Way anova followed by post-hoc Bonferroni test ($P < 0.05$ considered statistically significant).

Results: We observed that in the cellular models used, that mitochondria are depolarized and there is a reduction in the expression of the Pgc1 alpha gene in addition to a decrease in oxygen consumption. These changes are also associated with

a decrease in ATP / ADP ratio and decreased cell viability. At the same time, we observe that MS-275 and SB were able to rescue the ATP/ADP ratio, restore mitochondrial membrane potential and restore cell viability.

Conclusion: We conclude that MS-725 and SB, through epigenetic modifications, have shown neuroprotective effects over oxidative stress and excitotoxicity cellular models, restoring mitochondrial membrane potential, ATP/ADP ratio and cellular viability, therefore these compounds show therapeutical potential for not only ALS, but for any disease where oxidative stress and excitotoxicity are an underlying cause.

P016-F | Role of Nrf2-Keap1 signalling pathway in hyperglycaemia induced mitochondrial dysfunction

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Background: Diabetic nephropathy (DN) affects approximately 20%-40% diabetes patients worldwide. We have previously shown that hyperglycemia induced oxidative stress leads to mitochondrial dysfunction, measured as dysregulated mitochondrial DNA (MtDNA) levels, which may lead to the development and progression of DN. Nrf2-Keap1 (nuclear factor erythroid 2-related factor 2-Kelch-like ECH-associated-protein-1) system protects the body against oxidative stress. We hypothesized that Nrf2 activation may have a protective role on mitochondria by combating hyperglycemia induced oxidative stress. As part of this we first need to set up an in-vitro system where we can induce mitochondrial dysfunction and subsequently modulate Nrf2-keap1 to determine if this has any protective effect

Materials and methods: Human embryonic kidney 293 cells were cultured in 5 mmol/L (NG) and 25 mmol/L (HG) glucose and in 5 mmol/L glucose + 20 mmol/L mannitol (OC) for 6 days. Cell counts were measured on day 2.4 and 6; total DNA and RNA was extracted. MtDNA copy number (MtDNA) was measured in the DNA using q-PCR.

Results: No significant difference was observed in cell number on day 2 across all the groups. On day 4 and 6, cell counts were significantly ($P < 0.01$, $P < 0.001$) lower for HG cells as compared to NG. Hyperglycemia had no effect on MtDNA on day 2. On day 4, MtDNA was found to be increased significantly ($P < 0.05$) in cells grown in HG as compared to cells grown in NG; on day 6 MtDNA of HG cells was approximately 1.4 times less when compared to NG.

Conclusion: The data indicate that hyperglycemia increases MtDNA, which may be an adaptive mechanism, as the failing mitochondria tries to compensate the stress by increasing mitochondrial biogenesis. Further experiments will be conducted to determine the effect on genes regulating mitochondrial biogenesis and Nrf2-Keap-1 system which will help in elucidating the role of Nrf2-Keap1 system in mitochondrial dysfunction.

Funding Source: Commonwealth Scholarship Commission, United Kingdom.

P017-F | Setting up an in-vitro model of diabetic retinopathy in order to test compounds to protect hyperglycaemia induced mitochondrial dysfunction

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Background: Diabetic retinopathy (DR) is a major cause of acquired blindness in adults and affects up to a third of all patients suffering from diabetes. Mitochondria are subcellular organelles involved in energy production containing their own DNA (mtDNA). We previously reported that circulating mtDNA is altered and damaged at different stages of DR and data from animal models shows that diabetes can cause retinal mtDNA damage. In the current study, our aim is to set up an in-vitro model system of DR to be used for future drug screening programs. We will grow cells in diabetic conditions and establish the time course of mitochondrial damage.

Material and methods: HEK293 cells were treated in 5 mmol/L vs 25 mmol/L glucose and a parallel osmotic control for 6 days. Total DNA was isolated from day 2, 4 and 6 of treatment and mtDNA content was determined. MtDNA damage through the relative amplification of a long and short fragment as well as metabolic profiling will also be investigated.

Results: A pilot study using HEK-293 cells is currently being conducted and will be replicated using ARPE19 cells which are representative of the retinal pigmented epithelium cells. Preliminary data from HEK293 has shown an increase in MtDNA content in day 4 ($P < 0.05$) with a parallel increase in viable cell count ($P < 0.05$).

Conclusion: Preliminary data using MtDNA as an indicator of mitochondrial dysfunction shows that exposure to hyperglycaemic conditions can alter mitochondria within 4 days. Future work will involve determining the exact time course of mitochondrial alterations following hyperglycaemia using a range of assays. This will provide us with an in-vitro model

with which to test strategies to prevent diabetes induced mitochondrial dysfunction.

Funding sources: MRC DTP full scholarship.

P018-F | Mitochondrial dysfunction induced by marine benthic dinoflagellate toxic compounds

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Background: Marine benthic dinoflagellates are associated with human health risks through different routes of exposure. The present study intends to investigate the effects of cellular toxic compounds produced by the main dinoflagellate toxic genera on rat liver mitochondrial energetic function and permeability transitory induction.

Materials and methods: Different concentrations of Gambierdiscus excentricus strain UNR-08, Ostreopsis cf. ovata strain UNR-01 and Prorocentrum lima strain UNR-03 DMSO extracts were incubated with isolated mitochondria. Mitochondrial energetic function was evaluated by membrane potential ($\Delta\Psi m$) fluctuations and by ATPase activity. Permeability transitory pore (mPTP) induction was evaluated by means of $\Delta\Psi m$ fluctuations associated with successive calcium additions, and mitochondrial swelling. Statistical analysis was performed using one-way ANOVA, followed by the Dunnett's post-test.

Results: The gathered results showed that 934 cells/mg prot of G. excentricus and 7143 cells/mg prot of both O. cf. ovata and P. lima negatively affect mitochondrial function, including by decreasing ATP synthesis-related $\Delta\Psi m$ variations. Moreover, considerable much lower concentrations of dinoflagellate extracts (117 cells/mg prot of G. excentricus, 1429 cells/ mg prot of O. cf. ovata and 714 cells/ mg prot of P. lima) caused mPTP-induced swelling in calcium-loaded isolated mitochondria.

Conclusions: Although all extracts tested presented mitochondrial toxicity, G. excentricus extract was more toxic than O. cf. ovata or P. lima. Our results clearly demonstrated an effect of G. excentricus, O. cf. ovata and P. lima toxic compounds on the mitochondrial function by mPTP induction, which may lead to mitochondrial failure and consequent organism toxicity.

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P019-F | Effect of genipin on the membrane potential of cardiac mitochondria under conditions of ischemia and hypertension in rats

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Uncoupling proteins (UCPs) are proton carriers located in the inner mitochondrial membrane dissipating the energy of the proton gradient. Activation of UCPs occurs under excessive ROS production and is suggested as protective mechanism. In particular, increased expression of UCP3 in the heart is observed in ischemia-reperfusion, as well as in hypertension. However, the question about the activation of proton conductive activity of UCPs under these conditions remains opened. We used genipin - inhibitor of UCPs activity - to study the membrane potential of mitochondria from ischemized heart of adult male rats (Wistar, 6 months) and the heart mitochondria of spontaneously hypertensive rats (SHR, 24 months).

Isolated hearts by the Langendorff preparation were underwent 20 min ischemia. The membrane potential of the mitochondria was measured using the trimethylphenylphosphonium (TPMP+) selective electrode and calculated by the Nernst equation.

Preincubation with genipin (10-5 mol/L) did not affect the membrane potential of intact mitochondria (-163.1 ± 1.53 mV, n = 4 vs −166.3 ± 1.15 mVl, n = 4, correspondingly), that may indicate a slight proton conductive UCPs' activity under normal conditions. Ischemia significantly decreased the value of the mitochondrial membrane potential -140.6 ± 4.9 mV (n = 6, $P < 0.006$). Preincubation of mitochondria of ischemic heart with genipin increased the membrane potential to 148 ± 4.4 mV as well as in SHR mitochondria 173.5 ± 3.1 mV (n = 7, $P < 0.001$) vs 160.6 ± 2.82 mV in SHR without genipin (n = 7), indicating the inhibition of UCPs-mediated proton leak.

Thus, our results indicate that the proton conductivity of UCPs increases in response to the activation of the oxidative metabolism in myocardial ischemia, as well as in hypertension, and genipine can be used as a specific inhibitor of UCP-mediated proton conductivity of mitochondrial membranes.

P020-F | Profiling of proteostasis, mitochondrial and redox markers in the brain and liver from the R6/2 Huntington's disease mouse model

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Huntington's disease (HD) is caused by a CAG expansion in the huntingtin gene. Huntingtin is expressed in all tissues and HD is increasingly recognized as a disease that affects not only the brain, but also the whole body. Altered proteostasis, mitochondrial dysfunction and oxidative damage are considered pathological hallmarks of HD, and this work aimed to study how mutant-Htt affects these hallmarks in different tissues of a HD mouse model. Brain and liver samples from 11-weeks control mice (B6CBAF1/J) and transgenic mice expressing mutant-Htt-exon-1 (B6CBA-R6/2; displaying motor phenotypes) were processed for protein extraction and analyzed by immunoassays. In the R6/2 cortex, Hsp70 levels were increased suggesting an activation of the heat shock response. Levels of the mitochondrial Hsp70 and of the Hsp40, which is a chaperone required for the formation of a disaggregase complex, were decreased. Levels of Hsp60, Hsp90 and Hsp110 were similar to control. The R6/2 cortex also presented increased levels of ubiquitinated proteins and decreased p62 levels, suggesting alterations in protein clearance pathways. Concerning mitochondria, the R6/2 cortex presented increased NRF1 and mitofusin 2, and decreased TFAM levels. In contrast to the cortex, the R6/2 liver presented no alterations regarding proteins involved in the proteostasis network or in mitochondrial functions. Regarding oxidative stress, while both tissues presented increased SOD2 levels, the liver also showed increased carbonyl content and reduced levels of NRF2 (involved in the antioxidant response). Summing up, these data indicate that R6/2 mice present oxidative damage in the liver, and altered proteostasis and mitochondrial dysfunction mainly in the cortex. These results suggest differential, tissue-dependent, pathogenic mechanisms and an interplay between mitochondrial dysfunction and altered proteostasis in brain pathogenesis.

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P021-F | Evaluation of the toxicity induced by Doxorubicin in different age male CD-1 mice

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Doxorubicin (DOX) is used to treat several types of cancers. It causes cardiotoxicity and several risk factors could aggravate it. The aim of this work was to study the toxicity of clinically relevant DOX doses in infant (4 weeks), adults (3 months), and old (18-20 months) male CD-1 mice. DOX-treated infant and adults received a total cumulative dose of 18 mg/kg, while a second group of adults and also old mice received 9 mg/kg. Biweekly intraperitoneal administrations, for 3 weeks were given to all animals. The animals' weight, food, and water consumptions and general welfare were assessed throughout the experiment. Mice were sacrificed 7 days (adults and old) or 17 days (infants) after the last drug injection. The organs (liver, heart and kidneys) were removed and weighted and blood was collected. The heart, kidneys, and liver were analyzed through light microscopy and plasma was used for biochemical determinations. Significant body weight loss and decreased food and water consumptions were observed in DOX-treated infant and adult 18 mg/kg, when compared to controls. The organ weight/ brain weight ratios were significantly decreased in the DOX-treated adult (18 mg/kg). In the DOX-treated adult (18 mg/kg), aspartate aminotransferase (AST) and creatine-kinase MB (CK-MB) plasma levels increased, while the biochemical parameters in DOX-treated adult (9 mg/kg) showed no differences. Regarding the histological observations, all the DOX-treated populations presented lesions in heart, kidneys and liver, as so the control elderly mice. DOX provoked lesions in the cardiac tissue, but cardiac damage was more evident DOX-treated adult 18 mg/kg than in 9 mg/kg. Higher cumulative dose and adult mice were key for DOX-induced toxicity in our work.

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P022-F | Mitoxantrone-induced toxicity in different age male CD-1 mice

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Mitoxantrone (MTX) is a chemotherapeutic drug currently used against cancer, causing severe side effects. The aim of this work was to study the toxicity of clinically relevant MTX in infant (4 weeks), adults (3 months) and old (18-20 months) male CD-1 mice. All MTX-treated groups received a total cumulative dose of 6 mg/kg given biweekly through intraperitoneal administrations, for 3 weeks. The animals' weight, food and water consumptions and general welfare were recorded throughout the experiment. Mice were sacrificed 7 days (adults and old) or 17 days (infants) after the last drug injection. The organs were removed and weighted and blood was collected. The heart, kidneys, and liver were analyzed through light microscopy and plasma was used for biochemical determinations. Significant body weight loss was observed in the adult population treated with MTX. In addition, food and water consumptions decreased in the infant and adult populations treated with MTX. Regarding organ weight/ brain weight ratios, the ratio of all the organs were significantly decreased in the adult population treated with MTX. In the MTX treated-elderly population, only the spleen/brain ratio decreased. Alanine aminotransferase (ALT) plasma levels were significantly increased in the MTX-treated infant and no other significant changes were observed. Regarding the histological analysis, all the MTX-treated populations presented lesions (cellular degeneration, interstitial inflammatory cell infiltration, and necrotic zones) in heart, kidneys, and liver, as so the control elderly mice. The age of the animal models should also be taken into account when assessing anticancer drug toxicity and in this work and experimental paradigm, adult mice seemed to be more prone to MTX-induced toxicity.

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P023-F | Warburg effect in queens with spontaneous mammary tumors

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Background: Discovered at almost 100 years ago, the Warburg effect describes the use of anaerobic glycolysis by cancer cells as the preferred pathway to obtain energy, even in normoxemia conditions. The Warburg effect has been reported in several oncological diseases in human medicine, and to a lesser extent, also in veterinary oncology. However, to our best knowledge, this effect has not been assessed in feline mammary tumors. The aim of this study was to evaluate the Warburg effect in queens with spontaneous mammary tumors.

Material and methods: Serum concentrations of glucose, fructosamine, lactate and lactate dehydrogenase (LDH) were determined in 30 queens with malignant mammary tumors, in six cats with benign mammary lesions, and in 10 healthy control queens. Serum glucose, fructosamine, lactate and LDH were measured using commercially available kits, following instructions of the manufacturer.

Results: Serum LDH was significantly higher in queens with malignant mammary tumors than in healthy controls

($P = 0.04$). Serum lactate was higher in cats with malignant tumors when compared with controls, but the difference was not statistically significant ($P = 0.09$). No significant differences in concentrations of glucose or fructosamine were observed between the three groups of animals.

Conclusions: Our results suggest that the Warburg effect might be implicated in carcinogenesis of feline spontaneous malignant mammary tumors. However, no significant changes in serum glucose or fructosamine concentrations were observed, thus a typical Warburg effect was not observed in our study. Further studies with a higher number of animals and with a prospective nature should be conducted to elucidate the clinical significance of the Warburg effect in feline mammary tumors. Moreover, future research should consider the changes in glucose metabolism as a potential therapeutic target in feline mammary cancer.

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P024-F | Changes in the OXPHOS proteome and alterations of mitochondrial parameters in fibroblasts of patients harboring defined mitochondrial disorders

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The aim of our studies was complex characterisation of mitochondrial respiratory chain function and the related parameters responsible or involved in mitochondrial defect-mediated cellular dysfunction in fibroblasts from patients with defined mitochondrial disorders

These studies were performed with the use of neonatal fibroblasts from healthy donors and from infants harboring different defined mitochondrial defects (mutations in MTND, SURF1, SCO2, MTATP6, DGUOK and PDHA1 genes).

Considering that oxidative stress represents an imbalance between ROS production and the cellular antioxidant defence system, we investigated both the level/production rate of cytosolic and mitochondrial superoxide and hydrogen peroxide in the examined fibroblast lines. The greatest increase in ROS production was detected in fibroblasts harbouring mutations in mtDNA genes encoding subunits of mitochondrial respiratory complex I or harbouring mutations in nDNA (in SCO2 or SURF1 genes). Interestingly, mitochondrial superoxide production was significantly increased in nearly all of the examined patients' fibroblasts. These result indicate the presence of oxidative stress in patients' fibroblasts. In all of the examined at this step cell lines harbouring a mitochondrial defect, the expression pattern of antioxidant enzymes was different from the control values. Particularly, a significant increase in the level of SOD1 was detected in fibroblasts harbouring a mutation in the MTND, MTATP6 or SCO2 gene.

In conclusion, detailed principal component analysis revealed that each examined group containing fibroblasts with the same defined mitochondrial defect could be characterised according to a unique profile of bioenergetic, ROS production, and antioxidant enzyme expression pattern parameters. Using two components (PC1 and PC2), it was possible to detect differences between the examined fibroblast lines.

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P025-F | Profiling the markers of oxidative stress in post-mortem human brain samples

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Confirmation and understanding the mechanism of mitochondrial disorders very often is based on the proteomic studies showing decreased level or the lack of certain proteins or respective subunits what can explain observed clinical phenotype. In the optimal situation freshly collected biopsy or cultured fibroblasts from living patients should be used, however in many cases diagnosis of mitochondrial disorders could be performed only post mortem.

To study mitochondrial respiratory chain profile, antioxidant defense system profile as well as manifestation of the oxidative stress (protein carbonylation, protein nitration and DNA damage), nine individuals (six patients with defined mitochondrial disorders harboring mutations in MTATP6, SCO2, MPV17 and POLG genes; two patients who died due to the congenital heart defect and one patient with haemophagocytic syndrome) were studied.

We evaluated manifestation of oxidative damages and the pattern of intracellular oxidative defense system to in order to check whether such post-mortem material can be used to show the abnormalities in the respiratory chain and oxidative stress-related damage of proteins, DNA and lipids. Notably, there was no clear dependency between the oxidative stress markers and the presence of mitochondrial dysfunction.

In conclusion, this comprehensive and comparative study proves that human post-mortem brain samples (the basal ganglia with the level mammillary bodies), have poor diagnostic value, either to support or exclude mitochondrial disorders based on manifestation of the oxidative stress. The results have clinical implication. Indeed, in the optimal situation when brain autopsy is used for evaluation of proteome abnormalities, if possible the samples from patients should be also matched according cause of death (especially taking in to consideration fast-death and slow-death cases).

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P026-F | Mitochondrial HER2 stimulates respiratory chain function and drives tumorigenicity of breast cancer cells

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Amplification of HER2/ERBB2, a breast cancer oncogene, leads to resistance to therapy and poor prognosis in breast cancer patients. HER2, a member of the EGFR protein family, normally localizes to the plasma membrane. Recently, a fraction of HER2 has been reported at the inner mitochondrial membrane, where it interacts with the respiratory chain. Now we show that overexpression of mitochondrial HER2 (mtHER2) stimulates respiratory chain function in breast cancer cells in a tyrosine kinase-dependent manner. Moreover, overexpression of mtHER2 increases proliferation, migration and reactive oxygen species production. Interestingly, mtHER2 makes the cells prone to treatment with mitochondria-targeted tamoxifen (MitoTam), a new respiratory complex I inhibitor now in clinical trials. Therefore, mtHER2 affects both tumorigenicity and sensitivity to treatment by regulating mitochondrial bioenergetics and represents therefore a new treatment opportunity in HER2 high breast cancer.

P027-F | Human pheochromocytoma cancer cells as a plausible model of mitochondrial complex II-linked tumorigenesis

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Pheochromocytoma and paraganglioma (PHEO/PGL) are neuroendocrine tumours that are frequently associated with mutations in mitochondrial complex II (CII) subunits SDHA-D. Clinical data indicate that SDHB-deficient PHEO/PGL are less proliferative but are associated with higher invasiveness and metastasis. We have previously shown that both the loss of SDHB and/or depletion of mitochondrial (mt)DNA in the breast cancer cell line MDA-MB-231 leads to alternative assembly of CII_{low}, which maintains homeostatic control of metabolite synthesis under bioenergetics stress and supports tumour growth. However, PHEO/PGL malignancies arise from the chromaffin tissue; it is therefore expected that the epithelial-derived cells, such as breast cancer cells, are not an optimal model to decipher the consequences of CII mutations in PHEO/PGL.

For this reason, human chromaffin-derived pheochromocytoma cancer cells line hPheo1 was used to introduce SDHB deficiency (SDHB KO cells) by gene manipulation. PGL tissue, mitochondria, and whole cell lysate were used for respiratory assay, native blue gel electrophoresis (NBGE) and western blotting.

Similar to patient-derived SDHB-deficient paraganglioma tissue, SDHB KO cells featured substantial CII_{low} in the absence of fully assembled CII on NBGE, accompanied by prominent decrease of respiration and other relevant alterations. Further analysis of this unique model is ongoing.

Our data provide a new insight into pathological features of hard-to-treat SDHx-related PHEO/PGL. We suggest that the existence of CII_{low} in patients with SDHB-mutated paraganglioma substantially contributes to tumorigenicity, and interference with CII_{low} could ameliorate severe pathological outcomes, including enhanced migration and invasiveness.

P028-F | High-resolution respirometry for mitochondrial function analysis in skin of diabetic rodents

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Background: Chronic diabetic foot wounds (DFU) is a serious complications of diabetes, and the main cause of non-traumatic amputation of the lower limbs. Thus, understanding the mechanisms that lead to DFU in diabetes is very important to improve the quality of life of patients. Purpose: Evaluate mitochondrial respiration of the skin of rodents in the presence or absence of diabetes.

Materials and methods: Skin samples were collected from Wistar rats, as well as diabetic and non-diabetic C57/BL6 mice. Small skin biopsies were permeabilized with saponin (5 mg/mL). Mitochondrial respiration was assessed using 2 high-resolution respirometry protocols (Oxygraph-2k; Oroboros, AU). Briefly, in P1, leak respiration was determined after the addition of pyruvate (P, 5 mmol/L), malate (M, 2 mmol/L) and glutamate (G, 10 mmol/L). Electron transfer was coupled to phosphorylation by the addition of saturated concentrations of ADP (5 mmol/L). Addition of cytochrome C (10uM) was done to evaluate the integrity of the outer membrane of mitochondria. Succinate (10 mmol/L) was then added to evaluate the contribution of complex I and II to coupled respiration. In P2, octanoyl carnitine (0.5 mmol/L) and M (2 mmol/L) substrates were added to measure leak. Then ADP (7.5 mmol/L) was added to evaluate beta-oxidation and then P (5 mmol/L), G (10 mmol/L) and cytochrome C were added as above. Finally, FCCP (0.5 mmol/L) was added to measure maximal uncoupled respiration.

Results: Leak respiration were similar between the two protocols and groups. Coupled respiration was significantly lower in P2 ($P < 0.05$ for S, R, and O) in diabetic mice compared to non-diabetic mice.

Conclusions: The optimization of respiration showed robust and stable oxygen fluxes in both protocols. This technique proves useful for assessing mitochondrial respiration in the skin in a small amount of tissue, with possible application in the identification of the state of mitochondrial alterations in tissues under diabetes conditions.

P029-F | Diet-induced high cardiovascular risk impairs brain hippocampal metabolism and long-term spatial memory in middle-aged male rats

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Background: Cardiovascular disease is the major cause of disability and death in middle-aged men, and a socioeconomic concern. Patients are often hypertensive, dyslipidemic, diabetic and obese - all associated with modern lifestyle and aging. Their coexistence renders patients to high cardiovascular risk (HCR) and the worst prognosis, exacerbating brain/cognitive damage and the risk for Alzheimer's disease (AD). However, the crosslinking mechanisms are unknown. We hypothesized that HCR at midlife affects brain and cognitive performance leading to AD.

Materials and methods: We aimed to analyze the effect of diet-induced HCR on cognition, brain metabolic and AD markers in middle-aged (8-month-old) male Wistar rats, fed (or not) on salted diet and spontaneously-hypertensive rats (SHR), by determining peripheral blood parameters, cAMP, cholesterol and phospho-Tau in hippocampal lysates, and spatial memory through the Morris watermaze (MWM) test.

Results: Blood pressure, insulin levels and HOMA-IR were higher in Wistar rats fed on salted diet than in controls, pointing to hypertension and insulin resistance. Their body weight, systolic blood pressure, glycemia, HbA1C and total cholesterol were similar to SHR rats. They also took longer to reach the MWM platform, crossed less the swimming-pool, spent less time in the right quadrant and more in the opposite one than SHR rats, suggesting a deficit in their long-term, hippocampal spatial memory. They also had lower brain hippocampal cAMP, cholesterol and phosphoTauThr181 (related with earlier AD stages) than SHR rats.

Conclusions: Diet-induced HCR may inhibit middle-aged male rat brain metabolism, impairing long-term spatial memory. This issue deserves further clarification.

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P030-F | Resistin blunts neutrophil migration into atherosclerotic plaques: a possible stabilizing role

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Background: Adipocytokines regulate neutrophils during atherogenesis. However, the specific effect of resistin on neutrophil functions in atherosclerotic patients remains elusive. Here, we investigated the relationships between serum levels of resistin and neutrophil products in the systemic circulation and within plaques in a cohort of patients with severe carotid plaque stenosis undergoing endarterectomy. Furthermore, to investigate the molecular mechanisms underlying the observed results we assessed the effect of resistin on neutrophil pro-atherosclerotic functions in vitro.

Material and methods: Inflammatory biomarkers, neutrophil products and resistin levels were assessed in patients' sera and carotid plaques by ELISA and immunohistochemistry analysis. In vitro, human primary neutrophils isolated from healthy donors were assessed on different substrate cultures for: degranulation (by ELISA), migration (by microchemotaxis Boyden chamber), F-actin polymerization (by fluorescent assay), integrin and chemokine receptor expression (by flow cytometry) and apoptosis (by both morphologic analysis and flow cytometry).

Results: In atherosclerotic patients, plasma resistin levels positively correlate with those of different neutrophil granule products. Oppositely, resistin negatively correlates with neutrophil and MMP-9 plaque contents. In vitro, resistin is found in supernatants of degranulating neutrophils and positively correlates with other granule products. Resistin do not affect neutrophil degranulation, apoptosis and expression of integrin or chemokine receptors. Interestingly, pre-incubation with human recombinant resistin abrogates neutrophil migration towards the proatherosclerotic chemokines CXCL8 and polymerization of F-actin via ERK2 phosphorylation inhibition.

Conclusions: Resistin is released by degranulating neutrophils and blunts their infiltration into atherosclerotic plaques by blunting the migration towards known atherosclerotic mediators. These results suggest resistin as a potential immuno-modulator in the context of atherosclerotic plaque inflammation.

P032-F | The role of circulating miR-424 as marker of disease severity in pulmonary hypertension

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Background: Pulmonary hypertension (PH) is a severe progressive cardiopulmonary disorder that carries a high mortality rate, for which there is no cure at this time. It has been shown that dysregulation of Smurf1 and dysfunctional gap junction intercellular communication are implicated in cardiovascular of diseases. Although several studies have suggested that changes in miRs profile are implicated in PH, the underlying molecular mechanisms, including the identification of targets, remain largely elusive. In this study, we investigated whether circulating miR-424 can be used as biomarkers in PH, as well as the signaling pathways modulated by this miRNA.

Material and methods: Circulating miR-424 was evaluated by qRT-PCR. The levels of SMURF1 and Cx43 were assessed by western blot and structural changes in the right ventricle evaluated by Transmission Electron Microscopy (TEM).

Results and conclusion: The levels of circulating miR-424 are increased in PH patients when compared with healthy subjects, and correlated with decreased cardiac output. We showed that hypoxia induces the secretion of miR-424 by PAECs, which after being taken up by cardiomyocytes leads to downregulation of SMURF1. In the monocrotaline rat model of PH, we found an association between circulating miR-424 levels, the stage of right ventricle (RV) hypertrophy, and SMURF1 levels. Moreover, we found a modulation of Cx43 levels in the hypertrophied RV. This study shows that miR-424 has diagnostic and prognostic value in PH patients, correlating with markers of disease severity. Additionally,

miR-424 can target proteins with a direct effect on heart function such as SMURF-1, suggesting that this miRNA can act as a messenger linking pulmonary vascular disease and right ventricle hypertrophy.

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P033-F | The heart rate spectral analysis in patients with chronic heart failure

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Background: Chronic heart failure (CHF) is a widespread pathology of the cardiovascular system, leading to the life-threatening complications. Heart rate variability (HRV) indices contain important information for their predicting.

Materials and methods: We analyzed 24-hour ECG records obtained for patients with progressive CHF, including 23 persons with preserved ejection fraction and end-diastolic size of the left ventricle EDS <58 mm, and 45 persons with reduced ejection fraction and dilation of the left ventricle (EDS ≥ 58 mm). The spectral analysis of 5 minute fragments corresponding to different forms of patient activity has been performed.

Results: The power fractions in the ranges from 0.04 Hz to 0.15 Hz (LP) and from 0.15 Hz to 0.4 Hz (HP) were significantly higher in patients without left ventricle dilation (group 1) than in patients with reduced ejection fraction and the left ventricle dilation (group 2) for the HRV records corresponding to morning awakening and slow walking ($P = 0.03$); the ratio of LP to total power was also higher in group 1 in morning awakening ($P = 0.03$).

Conclusion: Our results indicate that HRV spectral indices differ significantly depending on the presence of dilation and could be used as complementary indicators in the CHF and the dilation status prediction.

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P034-F | Latent myogenic trigger points effects on upright stance

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Previous studies showed that dizziness can be caused by dysfunction of the deep muscular proprioceptors in the upper cervical spine leading to abnormal input to the vestibular nuclei. We investigated how latent myogenic trigger points (lMTP) of cervical muscles can affect postural stability.

In this study 79 volunteers 21-22 year old in average with latent trigger points of cervical muscles (39M/40F) participated. Control group represented by 28 individuals (10M/18F). All individuals were questioned on previous history of disease and underwent neurological assessment to exclude central nervous system, vestibular and other non-cervical causes of the dizziness. To evaluate postural stability we used force plate standard test within 60s. All individuals were evaluated once and in equal terms. The following rates had been analyzed: sway area, anterior-posterior and medial-lateral sway, balance quality rates, mean linear and angular velocities. All subjects with latent trigger points were divided into 2 subgroups: (a) individuals with 1 to 3 affected muscles (b) and individuals with more than 4 affected muscles. Individuals of second group showed significant deterioration of majority of posturographic rates in comparing with first group and control group. Interestingly, 1st group subjects represented relevant improvement of majority analyzed parameters.

As sensory information from the neck is combined with vestibular and visual information to determine the position of the head on the neck, and space we suppose that in cervical vertigo could occur when neck input became dominant over vestibular in subjects with lMTP in 4 and more cervical muscles, meanwhile 1-3 lMTP increase postural stability through neck stabilization. The reported study was funded by RFBR according to the research project №18-315-00263.

P037-F | Circulating exosomal connexin-43 as a potential biomarker for myocardial infarction

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Background: Intercellular communications are involved in numerous cardiac physiological and pathophysiological processes. Regarding their mediators, extracellular vesicles,

namely exosomes, seem to play a key role in both myocardial regeneration and repair. Connexin-43 (Cx43), an abundant transmembrane protein, is to be found in cardiac exosomes and its serum presence has recently become measurable. The aim of this study is to explore the potential of serum exosomal Cx43 as an acute myocardial infarction (AMI) biomarker.

Material and methods: Prospective single-center study comprising patients admitted into a Cardiac Intensive Care Unit, presenting with AMI, in whom serum exosomal Cx43 was measured by enzyme-linked immunosorbent assay within 12 hours after admission. Demographic, clinical, laboratory, echocardiographic, angiographic and prognostic data were collected and related to Cx43 levels. Quantitative variables were assessed via Spearman correlation, whereas for categorical ones Mann-Whitney and Kruskal-Wallis tests were employed. All statistical analysis was performed using SPSS version 23 (IBM Corp., Armonk, NY, USA).

Results: 28 patients were included. Mean age was 65 ± 11 years and 18% were female. All patients presented with ST-segment elevation myocardial infarction and were found to have obstructive coronary artery disease by means of emergent coronary angiography. Median GRACE score was 126 and none of the patients died during hospitalization. Serum exosomal Cx43 levels were higher in females ($P = 0.033$) and appeared independent of patient age and body mass index. Besides, they were not related with disease severity, as evaluated by GRACE score, Killip-Kimball class, maximum serum troponin, number of afflicted vessels and left ventricle ejection fraction, size and wall thickness. In addition, no association with classic risk factors, such as diabetes mellitus, hypertension, chronic kidney disease and anemia, was identified.

Conclusion: Serum exosomal Cx43 seems of little value as an AMI biomarker.

P038-F | Direct oral anticoagulants (DOACs) adjustment in patients with cardiac implantable devices and non-valvular atrial fibrillation: A single-centre retrospective study

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Background: Direct oral anticoagulants (DOACs) are the first line therapy for stroke prevention in non-valvular Atrial Fibrillation (NVAF). Observational studies are evidencing widespread discordance between guidelines and real-world practice regarding DOACs doses. Cardiac implantable devices improve the diagnosis and monitoring of NVAF patients where anticoagulant control is required. Therefore, real-world studies in this population can contribute to a better understanding of this off-label dose use and the impact in safety and efficacy outcomes.

Material and methods: A single-centre observational retrospective study was performed enrolling all patients implanted with a cardiac device between January and September of 2011 in Cardiology Department of Coimbra Hospital and Universitary Centre (Portugal). Therefore, 61 patients with NVAF and taking DOACs since 2011 up today were included in the study. Baseline demographic and clinical characteristics were evaluated and the adjustment according EMA guidelines was assessed, considering DOACs switch.

Results: In this cohort, mean age was 83.4 ± 8.5 years and 65.5% of the patients were male, with a mean body mass index of 29.2 kg/m². Among these patients, 16 have switched DOAC during follow-up, of which 14 switched one time and 2 switched two times, so 73 dose adjustments were considered for analysis. In this context, approximately 63.0% of the patients received doses in accordance to the European guidelines. However, 20.6% of the patients received underdosed off-label doses while 16.4% were overdosed. Dabigatran was the DOAC most frequently underdosed and edoxaban was the most frequently overdosed.

Conclusions: Among NVAF patients with implantable devices under DOAC therapy herein included, 37.0% of them were administered with off-label doses which can lead to an increased risk of stroke, bleeding and/or adverse effects. Other factors besides renal function, age and weight (e.g. increased bleeding risk and concomitant drugs) must also be assessed.

P039-F | Higher plasma GlycA, a novel pro inflammatory glycoprotein biomarker, is associated with reduced life expectancy: The PREVEND study

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GlycA is a novel pro-inflammatory marker that identifies N-acetyl-glycan groups mostly attached to acute phase

glycoproteins. We determined effects of GlycA and high sensitivity C-reactive protein (hsCRP) on life expectancy (LE) in men and women of the Prevention of Renal and Vascular End Stage Disease (PREVEND) cohort. GlycA and hsCRP were determined in 5,526 subjects. LE was compared in the upper quartile of both GlycA and hsCRP vs. the respective lower three quartiles combined, adjusted for LE of individuals in the Dutch general population of the same birth cohort and sex. Median follow up was 8.5 years [interquartile range 7.9–9.0], during which 348 (6.3%) subjects had deceased. LE at the end of follow up was lower in the highest vs. the lower three quartiles of GlycA ($P < 0.001$) and hsCRP ($P < 0.001$). Both men as well as women in the highest GlycA quartile had reduced LE vs. the lowest three quartiles combined ($P < 0.001$ and $P = 0.02$). For hsCRP, this was only observed in men ($P < 0.001$) but not in women ($P = 0.67$). This population-based cohort study demonstrates that higher plasma levels of GlycA were associated with reduced LE in men and women. With regard to hsCRP this only applied to men.

P040-F | Development and validation of the first HPLC method for quantification of perampanel and stiripentol in mouse matrices using an innovator salting-out assisted liquid-liquid extraction procedure optimized by a design of experiments approach

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Background: Perampanel and stiripentol are third-generation antiepileptic drugs approved as add-on therapies. Due to recent introduction in market, their applicability could be extended being necessary to perform more pharmacokinetic studies in rodents using a quantitative bioanalytical method. So, our aim was to develop and validate an HPLC method for simultaneous quantification of perampanel and stiripentol in mouse matrices using a simple and fast salting-out assisted liquid-liquid extraction (SALLE) procedure optimized employing a quality by design approach.

Material and methods: 100 µL of sample (plasma, brain, liver or kidney homogenates) were spiked with terbinafine (internal standard) and extracted by SALLE using 100 µL of 1M MgSO4 solution and 200 µL of isopropanol. MgSO4 volume and concentration and isopropanol volume were optimized using a response surface methodology based on central composite design of experiments (DOE) using MatLab. Compounds were separated using a LiChroCART® Purospher Star column (C18, 55 × 4 mm; 3 µm) at 45°C and a mobile phase [1% triethylamine in water pH 2.5/acetonitrile (57:43, v/v)] isocratically pumped at 1 mL/min. Fluorescence detection of stiripentol and terbinafine was performed at 254/372 nm and perampanel at 275/430 nm. Preliminary pharmacokinetic studies were carried after oral administration of perampanel and stiripentol to mice.

Results: This method was validated according to international guidelines being linear for perampanel over the concentration range of 1-500 ng/mL in brain, 2-2000 ng/mL in liver and 1-2000 ng/mL in plasma and kidney, and being linear for stiripentol between 10-2000 ng/mL in brain and 10-20 000 ng/mL in the remaining matrices. Method showed to be precise (CV ≤ 15%) and accurate (bias ± 15%) and the recoveries were in accordance with those predicted by DOE. Its applicability was demonstrated through the preliminary pharmacokinetic studies.

Conclusions: This method was successfully validated and applied suggesting to be a simple and fast tool to support future non-clinical pharmacokinetic-based studies involving perampanel and stiripentol.

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P071-F | Monocyte and not Neutrophil derived microvesicles are able to activate endothelial cells and smooth muscle cells and to enhance recruitment of PMN in a Flow Chamber based assay

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Released from the plasma membrane of any cells, microvesicles (MVs) are emerging as novel effectors of cell-to-cell communication in inflammation. Beside expressing common markers of the cell of origin, MV differ in size, macromolecular and biological composition in relation to the mode of cell activation. This heterogeneity raises the question as to whether MV subtypes might be new tools for diagnosing diseases. The aim of this study is to investigate human monocyte and neutrophils-derived MV heterogeneity and function in vitro thus mimicking vascular inflammation.

$1x10^6$ monocytes isolated with Rosette Sep™ technology or $20x10^6$ neutrophils isolated by density gradient separation were

stimulated for 1 hours with TNFα (50 ng/mL) and MV profile was studied with Imaging flow-cytometry. Human umbilical vein endothelial cells (HUVEC) or human vascular smooth muscle cells (hVSMC) were incubated with different MV subsets at a ratio of 10:1 MV:cell for 24 hours, and expression of adhesion molecules (ICAM-1, VCAM-1, CD62E) on HUVECs and calcification in hVSMCs was quantified. HUVEC response was investigated further using flow-chamber assay.

MV analyses identified in monocyte-derived MV samples presence of platelets MV ($CD41^+/CD14^-$), monocytes MV ($CD41^-/CD14^+$), as well as a subset bearing both markers ($CD41^+/CD14^+$). Functional studies showed that TNFα-stimulated monocyte-derived MVs enhanced expression of VCAM-1, ICAM-1 in HUVECs. Activation of hVSMC through calcification was also demonstrated. 24hs stimulation of HUVECs with the TNFα-stimulated monocyte-derived MV in flow-chamber assays showed a reduction in rolling velocity associated with increased numbers of adherent and emigrated PMN. In all cases, neutrophils-derived MVs were less active.

We have identified heterogeneous populations of MV derived from isolated monocyte with incidence of monocyte MVs, platelet MVs and a double positive MV subset. Since only these MVs promoted HUVECs and hVSMCs activation, we propose that monocyte-platelet-derived MV can perpetuate vascular inflammation and might be used as diagnostic marker for vascular inflammation.

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P072-F | Effect of the molecular complex of pectin with acetylsalicylic acid on the leukocyte profile of rats with carrageenan paw edema

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NSAIDs are effective against pain, swelling and increased temperature, i.e., the symptoms that accompany many diseases, so they are widely used in the clinic. The main targets of NSAID are cyclooxygenase enzymes COX1 and COX2, inhibition of which leads to decrease in the production of prostaglandin inflammatory mediators. In addition, when using NSAIDs, a moderate immunosuppressive effect is revealed.

The aim of the work was to study the effect of the molecular complex of pectin with acetylsalicylic acid (hereinafter PASA) in comparison with acetylsalicylic acid (hereinafter ASA) on the leukocyte profile of rats with carrageenan edema.

The study was performed on 28 adult males of Sprague Dawley rats. To simulate an inflammatory reaction, 100 μL of 1% aqueous carrageenan solution was administered under plantar aponeurosis of the right hind paw in rats. After 30 minutes, the studied drugs were orally administered once in the form of aqueous solutions: ASA 10, 20 and 40 mg/kg, PASA 100, 200 and 400 mg/kg, water in the control group.

The analgesic effect of PASA at all doses is comparable to ASA at equimolar doses. The anti-edema effect of PASA at the dose of 400 mg/kg is similar to the effect of 40 mg/kg of ASA. Under the action of drugs, the slowdown of the growth of total number of leukocytes as well as changes in ratio of phagocytic cells: increasing of monocytes and decreasing of granulocytes number compared with the control, were shown. The most complete recovery of the leukocyte profile within 24 hours with the administration of 400 mg/kg of PASA was observed. The administration of 40 mg/kg of ASA and 400 mg/kg of PASA prevents an increase in interleukin 8, and 400 mg/kg of PASA - also in interleukin 6.

The study was funded by RFBR according to the project № 18-013-01177.

P073-F | A fluorescence-based method for studying the fusion of osteoclasts and myeloid multinuclear giant cells

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Background: Cell fusion (the fusion of precursor cells to a single cell with multiple nuclei) is a crucial step of several biological processes, such as the development of two myeloid-derived cell types: the osteoclasts and the multinuclear giant cells. Our aim was to develop a method for monitoring the fusion of these cell types by fluorescence microscopy.

Material and methods: We co-cultured bone marrow-derived myeloid progenitors of two genetically modified mouse strains. One of them constitutively expresses the tdTomato red fluorescent protein but switches to EGFP expression upon Cre-mediated recombination. The cells of the other strain express the Cre recombinase either constitutively (Rosa26-Cre) or in an osteoclast-specific manner (CtsK-Cre). Therefore, we expected the emergence of green fluorescence upon cell fusion. Fusion was triggered

by differentiation towards osteoclasts or multinuclear giant cells in vitro. The lack of fusion was modelled using macrophage cultures. Cellular differentiation and the appearance of green fluorescence were observed with fluorescence microscope. Recombination was also assessed at the DNA level by PCR.

Results: Beside the red fluorescence initially present, we could observe the emergence of green fluorescence in cultures where cell fusion was triggered. Based on phase-contrast images and nuclear staining, this corresponded to fused multinuclear cells. No green fluorescence was observed in cultures where no cell fusion was induced. Rosa26-Cre cells resulted in more intense fluorescence in osteoclast cultures than the CtsK-Cre ones, and the latter cells failed to induce green fluorescence when co-cultures were differentiated to multinuclear giant cells. The presence of the recombined allele could be confirmed in osteoclast cultures with PCR and was absent in macrophage cultures.

Conclusion: Our method allows us to continuously monitor the multinucleation process of fused cells in vitro, opening the possibility to assess the effect of pharmacological inhibitors and transgenic mutations on myeloid cell fusion.

P074-F | Src family Kinase-mediated vesicle trafficking is critical for neutrophil basement membrane penetration during acute inflammation

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Background: Leukocyte recruitment into inflamed tissue needs to be tightly regulated for an appropriate, but not excessive immune response. The process is following a cascade of adhesion and activation events and is highly dependent on leukocyte integrins which mediate extensive signaling activity in large part through Src family Kinase (SFK)-dependent signaling pathways.

Material and methods: We aimed to investigate the function of SFKs in neutrophil recruitment in vivo using intravital microscopy of inflamed cremaster muscle venules in Hck-/- Fgr-/- Lyn-/- mice lacking SFKs expressed in neutrophils. We visualized basement membrane digestion and penetration by wild type and SFK depleted neutrophils using spinning disc confocal microscopy.

Results and conclusion: SFK-ko mice exhibited severely reduced neutrophil adhesion due to defective postarrest modifications compared to control mice. Integrin clustering as well as activation of adhesion relevant proteins were strongly diminished, resulting in the inability to withstand shear forces exerted by the flowing blood. Interestingly, SFK deficiency also led to impaired basement membrane penetration which was due to reduced Rab27a-dependent surface mobilization of intracellular vesicles containing VLA3, VLA6. In addition, SFK-ko neutrophils also were unable to digest basement membrane components and thus hindering their extravasation. Taken together, our study provides strong evidence for a role of SFKs in neutrophil postarrest modifications and extravasation during inflammation. These findings may further support the current efforts to test SFK inhibitors in inflammatory diseases with unwanted neutrophil recruitment.

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P075-F | Novel insight into the role of the S100A8/A9 protein complex in the regulation of neutrophil functions

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S100A8 and S100A9, members of the S100 family of cytoplasmic EF-hand calcium-binding proteins, are abundantly expressed in the cytosol of neutrophils and mostly found under heterodimeric form. S100A8/A9 have various intracellular and extracellular functions and our previous results showed that their intracellular activity is carried by the phosphorylation of S100A9. Based on these results, we investigated the importance of S100A9 phosphorylation on the extracellular activity of the protein complex and its impact on pro-inflammatory functions of neutrophils. First, we analyzed the phosphorylation state of secreted S100A8/A9 and the mechanism by which the protein complex is released into the extracellular space. Our results show that S100A9 is secreted under a phosphorylated form within the S100A8/A9 complex and this release is highly correlated to NETosis. Next, we investigated the inflammatory response of neutrophil-like dHL-60 cells when stimulated with the phosphorylated and non-phosphorylated form of S100A8/A9. Our results indicate that only the phosphorylated form of S100A8/A9 increases the expression and secretion of various cytokines (e.g. TNFa, CCL4, CXCL8). Using receptor-neutralizing antibodies, we provide evidence that S100A8/A9-P is inducing cytokine secretion through TLR4 signaling. Finally, we investigated the

post-transcriptional response induced by S100A8/A9-P stimulation. Using miRNA-sequencing on S100A8/A9-P stimulated dHL-60 cells, we identified an upregulation of miR-146a-5p, miR-146b-5p and miR-155-5p expression. Since these three microRNAs have previously been described to regulate TLR4 signaling at various levels, we investigated their influence on the inflammatory response mediated by S100A8/A9-P. Stable overexpression of miR-146a-5p and miR-155-5p in dHL-60 cells resulted in the reduced S100A8/A9-P-mediated secretion of cytokines through the inhibition of key players in the TLR4 signaling pathways. To summarize, our results give new insight into the pro-inflammatory functions induced by S100A8/A9-P in neutrophils and reveal the potential of the phosphorylated protein complex as a major regulator of inflammation in chronic inflammatory diseases.

P076-F | Lifelong exercise practice and inflammaging: master athletes cytokine response to acute exercise

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Background: Aging is often associated with inflammaging, a low-grade pro-inflammatory state that represents a significant risk factor for numerous diseases. On the other hand, exercise has been shown to exert an anti-inflammatory effect and could be an efficient counter-measure to either prevent or delay the onset of chronic diseases associated with inflammaging. In this context, master athletes with a lifelong practice of regular exercise training represent a unique model to study aging in the context of optimized behavior regarding active aging. This study analyzed the effects of aging and lifelong training on several pro- and anti-inflammatory cytokines, and the impact of acute exercise on their expression.
Material and methods: Thirty-nine participants were allocated into 3 groups: young (31.8 ± 3.00 years), middle-aged (54.2 ± 5.9 years) and master athletes (53.1 ± 8.8 years) that performed a maximal incremental test on a cycle ergometer. Blood samples were obtained before (Pre), 10 min post-exercise (Post) and 1 hours post-exercise (Post 1 hours) and cytokines quantified by ELISA. Effects of age, training and age*training interaction were tested using two-way ANOVA, with Bonferroni multiple comparison post hoc testing.
Results: Mean VO2max was similar for master athletes and younger subjects and higher compared to the middle-aged group. At baseline, master athletes showed higher concentrations of IL-1ra, IL-1alpha and IL-8, while the highest values of IL-10 were observed in the younger group, with the middle-aged group showing the lowest values. IL-1beta increased following exercise in the younger, whereas IL-1beta and IL-6 decreased in the master athletes 1 hours Post (37.3%, 32.7% respectively) and IL-8 decreased in all groups. The TNF-alpha/IL-10 ratio was higher at all moments for the middle-aged group.
Conclusion: Lifelong training helps to maintain the balance of pro- and anti-inflammatory cytokines, and IL-10 levels close to those found in young adults.
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P077-F | Neutrophil extracellular traps mediated by the cytokine midkine critically promote cardiac inflammation in myocarditis

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Background: Myocarditis is a common cause of heart failure in young adults. While the role of the adaptive immune system in myocarditis has been widely acknowledged, the contribution of innate immunity and particularly neutrophils remains incompletely understood.
Material and methods: Sections of endomyocardial biopsies from patients with myocarditis or from mice after induction of experimental autoimmune myocarditis (EAM) were stained for neutrophil extracellular traps (NETs). Cardiac inflammation was assessed in mice with EAM after blocking NETs, the cytokine midkine (MK) or the low-density lipoprotein receptor-related protein 1 (LRP1).

Hoxb8-SCF cell-derived neutrophils (Hoxb8 cells) lacking LRP1 were used to study the role of the MK-LRP1 axis during neutrophil recruitment steps in microflow chambers in vitro. NET formation of Hoxb8 cells was investigated in the presence of MK.

Results: In this study, we identified NETs in the cardiac tissue of patients and mice with myocarditis. Targeting NETs during EAM substantially reduced cardiac inflammation. Inhibition of the cytokine MK attenuated NET formation and leukocyte infiltration, reduced fibrosis and preserved systolic function during EAM. Accordingly, blocking the MK receptor LRP1 with the receptor-associated protein resulted in diminished leukocyte infiltration during the acute phase of the disease and may therefore point to a role of the MK-LRP1 axis during EAM. In the inflamed cardiac tissue, MK was expressed in the perivascular compartment suggesting a direct contribution of MK to leukocyte recruitment. Using LRP1-deficient Hoxb8 cells we demonstrated that LRP1 represents the central receptor in MK-mediated neutrophil recruitment. Furthermore, MK triggered NET formation via LRP1 in Hoxb8 cells.

Conclusion: In summary, NETs critically contribute to the pathogenesis of myocarditis. In turn, MK drives cardiac inflammation by promoting neutrophil trafficking and NETosis. Thus, targeting MK or NETs may represent novel therapeutic strategies for the treatment of myocarditis.

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P078-F | Neutrophil effector functions are not impaired in duffy antigen receptor for chemokines (DARC)-null black South Africans

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Background: Neutrophil deficiency and/or dysfunction are associated with recurrent infections. The Duffy Antigen Receptor for Chemokines (DARC)-null genotype is predominant in African ancestry populations and is the major genetic determinant of benign ethnic neutropenia. DARC-null linked neutropenia is associated with an increased risk of HIV-1 acquisition and mother-to-child transmission. However, the impact of the DARC-null trait on HIV disease progression remains controversial. While the DARC-null genotype is associated with lower absolute neutrophil counts (ANCs), the effects of the polymorphism on neutrophil functions is unknown.

Methods: The impact of the DARC-null trait and lower ANCs on key neutrophil functions (proteolytic activity in the phagosome, reactive oxygen species (ROS) and neutrophil extracellular trap (NET) production) were assessed in 20 HIV negative and 22 HIV-1 chronically infected black South Africans. Proteolytic activity was measured by flow cytometry following FcR-mediated uptake of IgG opsonised beads. PMA activated neutrophils were measured for ROS production by chemi-luminesce and visualised by fluorescent microscopy for NET formation. Participants were genotyped for DARC using TaqMan allelic discrimination assays and ANCs were measured by full blood count.

Results and conclusion: The DARC-null polymorphism was highly prevalent in our cohort (69%) and was strongly associated with lower ANCs in uninfected ($P = 0.0007$) and HIV-1 infected ($P = 0.03$) subjects. Enhanced phagosome proteolytic activity was observed in the absence of DARC at 10 minutes ($P = 0.05$ and $P = 0.009$) and 60 minutes ($P = 0.05$ and $P = 0.07$) in uninfected and HIV-1 infected subjects respectively. ROS was unaffected by DARC trait irrespective of HIV status and the formation of NETs were only reduced in neutrophils from DARC-null HIV-1 infected individuals ($P = 0.04$) following prolonged stimulation. The data indicates differential neutrophil function in the absence of DARC that may be moderately modulated by HIV-1 infection. Overall the data suggests that the DARC-null trait is not deleterious to neutrophil functions in African populations.

P079-F | Identification of novel coronin 1a interacting proteins critical for neutrophil trafficking in innate immunity

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Background: During acute inflammation, neutrophils are recruited to sites of lesion by a tightly controlled multistep cascade, which critically relies on the spatiotemporal regulation of β₂ integrins (CD11/CD18). Recently, we identified coronin 1A (Coro1A) as a novel regulator of β₂ integrins that interacts with the cytoplasmic tail of CD18 and is

crucial for induction of neutrophil adhesion and postadhesion events. To decipher the underlying signaling pathway, we aimed at the identification of Coro1A interacting signaling partners and their role in neutrophil trafficking using the zebrafish model.

Material and methods: Co-immunoprecipitation was performed using neutrophil-like differentiated HL-60 cells stably expressing Coro1A-EGFP with the GFP-nanotrap® technique followed by mass spectrometry conducted in cooperation with Axel Imhof (Protein Analysis Unit, Biomedical Center, LMU, Munich). Co-immunoprecipitation experiments were performed with adherent cells upon exposure to immobilized fibrinogen in the presence of Mn^{2+} or in suspended cells, which were left unstimulated for control.

Results: In a screen for novel Coro1A interacting partners, we identified different candidate proteins involved in signaling or regulation of the actin cytoskeleton. A total of 22 proteins specifically interacted with Coro1A only in adherent cells, 30 proteins interacted only under unstimulated conditions and 43 proteins in adherent as well as suspended cells independent of β_2 integrin engagement. Proteins of interest are analyzed for their role in neutrophil function in vivo using the zebrafish line Tg(lysC:DsRed;fli1a:EGFP) with DsRed-labeled neutrophils and EGFP-labeled endothelial cells. Inflammation was induced by transection of the caudal fin fold. Three hours post wounding, neutrophils recruited to the wound were quantified by conventional light microscopy and migration velocity was measured using spinning-disk confocal microscopy.

Conclusions: The identification and characterization of novel Coro1A interacting proteins will provide a deeper understanding of neutrophil recruitment during innate immunity.

Funding sources: SFB914/A02.

P080-F | Natural pseurotins inhibit proliferation and inflammatory responses via the inactivation of STAT and ERK signaling pathway in murine macrophages

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Background: One of the bioactive natural compounds with high potential as pharmaceutical agents is pseurotin family. Pseurotins are a class of structurally unique yet underexplored bioactive natural products. Natural pseurotins are a secondary metabolite produced by many species of fungi, mainly by Aspergillus sp. During the pseurotin biosynthesis, a large number of closely related bioactive compounds, such as pseurotin D or synerazol are also formed. Natural pseurotins have antimicrobial and antiparasitic activity. Interestingly, a few studies suggested effects of pseurotins in eukaryotes, such as antiangiogenic activity.

Material and methods: Unstimulated and LPS-stimulated RAW264.7 macrophages were used for experiments. The effect of pseurotins on viability and cytotoxicity were measured by total amount of protein, LDH and MTT assay. Apoptosis were measured by caspase 3 and 8 activity. We measured mitochondrial respiration and glycolysis by Agilent Seahorse analyser. Phosphorylation of STAT and MAPK proteins and expression of cyclins were determined by Western blot.

Results: Natural pseurotins significantly inhibit metabolic activity and proliferation at the level of cyclins expression. Moreover, pseurotins affected LPS-activated macrophages. Significantly inhibited the NO production and IL-6 secretion in dose-dependent manner. On the other hand, TNF-α secretion did not affect by pseurotins. These results correlate with the results of known STAT inhibitors.

Conclusion: It can be concluded that natural pseurotins are able to inhibit proliferation, reduce oxidative stress, inhibit production of pro-inflammatory cytokines, NO and are able to modulate immune response. Therefore, they could represent a new group of drugs for therapeutic treatment associated with macrophages diseases.

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P081-F | Lc3-associated phagocytosis provides an intracellular niche for *Staphylococcus aureus* in neutrophils

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Background: *Staphylococcus aureus* is a major human pathogen capable of causing multiple pathologies ranging from cutaneous lesions to life-threatening sepsis. Although neutrophils have been shown to play a role in immunity against *S. aureus*, recent evidence suggests that neutrophils can provide an intracellular niche for staphylococcal dissemination. However, the mechanism of neutrophil subversion by intracellular staphylococci remains unknown. Targeting of intracellular pathogens by autophagy is recognised as an important component of host immunity, but whether autophagy is beneficial or detrimental to *S. aureus*-infected host remains controversial.

Materials and methods: Using a zebrafish model of staphylococcal infection accompanied by imaging of transgenic zebrafish, we explore the autophagic response to *S. aureus* within professional phagocytes.

Results and conclusion: We show that *S. aureus* becomes internalised by macrophages and neutrophils and is rapidly decorated by the autophagy marker Lc3. Upon phagocytosis by neutrophils, Lc3 positive, non-acidified spacious phagosomes are formed. This response is dependent on phagosomal NADPH oxidase (NOX2) as both p22phox knockdown and DPI treatment inhibited the Lc3 decoration of the phagosomes. Importantly, the inhibition of NOX2 diverted neutrophil *S. aureus* processing into tight acidified vesicles, which resulted in increased host resistance to the infection. Interestingly, intracellular bacteria within neutrophils were also tagged p62-GFP fusion protein, supposedly marking selective autophagy, with loss of p62 detrimental to the infected host.

Taken together, we have shown that intracellular handling of *S. aureus* by neutrophils is best explained by Lc3-associated phagocytosis (LAP), which appears to provide an intracellular niche for bacterial pathogenesis while the selective autophagy adaptor p62 is host-protective. Additionally, the observed antagonistic role of the autophagic machinery in *S. aureus* -infected neutrophils may explain the conflicting reports relating to anti-staphylococcal autophagy and provide new insights for therapeutic strategies against antimicrobial resistant staphylococci.

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P082-F | Exploring the pro-resolving characteristics of insoluble immune complexes in the context of neutrophil functions in humans

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Background: Immune complexes (ICs) are antigen-antibody aggregates that can activate neutrophils and induce a range of functions such as phagocytosis, generation of reactive oxygen species (ROS), production of cytokines and release of inflammatory mediators. Three types of ICs can be present in inflammatory conditions in the human body, soluble ICs, insoluble immune complexes (iIC) and immobilised ICs.

Phagocytosis-induced cell death (PICD) is a well-established phenomenon where internalisation of pathogens induces neutrophil apoptosis, promoting the resolution of inflammation. Our previous findings showed that iICs induce neutrophil apoptosis via a non-canonical pathway; hence we investigated whether iIC-induced neutrophil apoptosis is a form of PICD.

Material and methods: Freshly isolated neutrophils from healthy donor blood were stimulated with iICs or particles (IgG-opsonised zymosan or latex beads). (i) Induction of apoptosis was assessed at different time-points (0, 3, 6, 9, 12 and 24 hours) by flow cytometry and morphological analysis. (ii) Immunofluorescence was used to investigate internalisation of ingested particles. (iii) IgG degradation of internalised iICs and latex beads was analysed by Western Blot.

Results and conclusions: We show that (a) iICs and zymosan induce neutrophil apoptosis but IgG-opsonised latex beads do not. (b) Internalisation of beads and iICs depends on different signalling pathways and regulators, and the rate of internalisation is different. (c) Both internalisation events trigger IgG degradation. Chloroquine, an endocytosis blocking agent, prevents iIC degradation but not the degradation of IgG from latex beads.

iIC-induced neutrophil cell death and PICD are mechanistically distinct. Internalisation and degradation of iICs and opsonised latex beads are regulated by separate signalling pathways. iIC-induced neutrophil apoptosis might play a major role in the resolution of inflammation in autoimmune diseases.

Funding sources: Arthritis Research UK, Edinburgh Global Research Scholarship.

P083-F | Resolvins in human acute heart failure

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Background: Resolvins (Rvs) are inflammation resolving mediators that appear to attenuate cardiac and endothelial dysfunction in experimental studies, but were never explored in human acute heart failure (AHF). Thus, we aimed to evaluate Rvs, as well as their correlation with biomarkers of cardiac and endothelial injury/dysfunction, proinflammatory/redox status and prognostic scores in human AHF.

Material and methods: Patients with diagnosis of AHF (n = 10) and cardiogenic shock (CS) (n = 9) were included and blood samples were collected at days 1-2 (admission), days 3-4 and days 5-7. Blood donors were used as controls (n = 10). RvD1, RvE1, endocan, myeloperoxidase (MPO) and nitrotyrosine were measured with ELISA kits. C-reactive protein (CRP), B-type natriuretic peptide (BNP),

high-sensitivity troponin I (hs- trop I), APACHE II and SAPS II scores were also evaluated.

Results: At admission, RvD1 was significantly lower in CS than in AHF (CS vs AHF, $P = 0.024$; controls vs AHF or CS, $P =$ ns). RvE1 was higher in AHF and CS but was only significantly different in CS (controls vs CS, $P = 0.0041$; controls vs AHF or AHF vs CS, $P =$ ns). Both endocan and MPO were higher in AHF and CS groups at admission ($P < 0.05$ or $P < 0.001$ vs controls). There were no significant differences in the studied parameters when comparing all time points. Within patients, we observed significant correlations for RvD1 with endocan ($r = -0.38$, $P = 0.009$) or SAPS II ($r = -0.50$, $P = 0.029$), RvE1 with CRP ($r = 0.30$, $P = 0.047$) or MPO ($r = 0.29$, $P = 0.048$), endocan with BNP ($r = 0.51$, $P = 0.006$), nitrotyrosine with CRP ($r = 0.41$, $P = 0.006$) or hs-trop I ($r = 0.52$, $P < 0.001$) or MPO ($r = 0.80$, $P < 0.001$) and MPO with CRP ($r = 0.46$, $P = 0.002$) or hs-trop I ($r = 0.37$, $P = 0.018$).

Conclusions: RvE1 increases with clinical/hemodynamical severity in CS, being associated with proinflammatory status. RvD1 appears to be exhausted/inactivated in worse clinical scenarios in AHF spectra, being probably a protective mediator. [Funded by FCT/FEDER (COMPETE, Portugal 2020), PTDC/MEC-CAR/32188/2017].

P084-F | The role of augmenting material in *Staphylococcus aureus* interaction with macrophages

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Background: *S. aureus* lives within the polymicrobial microbiome and emerges from this to cause a wide range of serious human infections. We have recently found that co-infection with human skin commensals or even isolated cell walls (peptidoglycan), can augment *S. aureus* infection, leading to increased disease severity in murine models of infection (Boldock et al., 2018). During systemic infection in the mouse the macrophages, specifically Kupffer cells, are a primary line of defence. Augmentation occurs within Kupffer cells in an NADPH oxidase dependent manner, leading to a reduction in reactive oxygen species (ROS), increased pathogen survival and subsequent proliferation that leads to abscess formation. We have also found that augmentation of *S. aureus* survival in human monocyte derived macrophages (MDMs) occurs by the addition of cell wall material.

Materials and Methods: Here we are using a combination of in vitro and in vivo assays to elucidate the molecular mechanisms that underpin the augmentation phenomenon. Bacterial cell killing assays in vitro have begun to establish how the augmenting material is able to protect *S. aureus* from the bactericidal effects of phagocyte offensive mechanisms. This is being correlated with how augmentation occurs in human MDMs. We have developed a time-lapse microscopy approach to study bacteria-phagocyte interactions from the individual bacterium to phagocyte population level, within an individual experiment.

Results and conclusions: This time-lapse imaging allows rare events within a population, such as abscess formation, to be monitored and to be correlated with the fate of individual bacteria. Our studies shed new light on how an opportunist pathogen such as *S. aureus* can initiate disease, from within a polymicrobial environment and highlights new potential avenues to reduce the burden of infection, with such important consequences for human health.

Funding sources: MRC.

P085-F | Soluble uric acid impairs β2 integrin-mediated neutrophil migration in acute gouty arthritis during renal failure

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Background: Hyperuricemia (HU), defined by elevated serum uric acid (UA) levels, is strongly associated with gouty arthritis. Gouty arthritis is triggered by the formation of monosodium urate (MSU) crystals leading to an acute inflammatory response. Despite profound HU, only a minority of patients with advanced chronic kidney disease (CKD) experience gout attacks, but why? We speculated that soluble UA (sUA) might exert immunomodulatory effects during sterile inflammation induced by MSU.

Methods: Alb-creERT2/Glut9lox/lox (ki/ki) or Alb-creERT2/Glut9lox/lox without active cre (+/+) mice were injected with tamoxifen and placed on a chow or high-fat diet with inosine. To induce gout, MSU crystals were injected either into air pouches or cremaster muscles. Rolling flux fraction, leukocyte adhesion were analyzed and extravasated leukocytes counted.

Neutrophils isolated from healthy individuals were pre-incubated with or without sUA prior to stimulation with

CXCL8. The expression of LFA-1, MAC-1 and mAB24 were quantified by flow cytometry. Transwell migration assays were carried out with neutrophils from healthy individuals and CKD patients.

Results: Ki/ki mice on chow diet with inosine developed HU, whereas ki/ki mice on a high-fat diet with inosine developed HU and CKD. Intravital microscopy revealed that HU with or without CKD increased leukocyte rolling velocity, but reduced leukocyte adhesion and extravasation towards MSU crystal-induced inflammation.

In blood neutrophils isolated from healthy individuals, sUA significantly reduced the expression of LFA-1 and MAC-1 compared to CXCL8-stimulated neutrophils alone. sUA diminished β2 integrin activation and hence impaired neutrophil migration towards CXCL8. An impaired migratory capability was also observed in neutrophils from CKD patients.

Conclusion: HU suppresses sterile inflammation by modulating neutrophil migration. This mechanism might be responsible for the unexpected low prevalence of gouty arthritis despite persistent HU in CKD patients.

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P086-F | CRP induces NETosis in heart failure patients with or without diabetes

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Aims: C reactive protein (CRP) is recognized as a biomarker of chronic, low-grade inflammation associated to vascular disorders. Lately, the role of neutrophils and neutrophil extracellular traps (NETs) have been investigated as a potential source of chronic inflammation, obliteration of small blood vessels and formation of atherosclerotic plaque. The primary objective of this study was to investigate NETs as a marker of inflammation in patients with symptomatic heart failure (HF) with or without type 2 diabetes mellitus (T2DM). The secondary objective was to examine the correlation between NETs and CRP in these patients.

Methods and results: We performed a small non-interventional study including patients with HF±T2DM, T2DM and healthy controls (HC) group. Serum contents of NETs and other inflammatory markers were measured by ELISA. The release of NETs (NETosis) in vitro by the neutrophils under various stimuli was measured by confocal microscopy. The levels of NETs in the serum of HF patients were significantly higher as compared to HC (106% increase; $P = 0.014$). Serum CRP concentrations were significantly increased in all 3 groups of patients (HF, T2DM and HF+T2DM) as compared to HC ($P \leq 0.03$), and a positive correlation was observed between serum CRP and NETs levels ($P = 0.03$). Neutrophils from HF and HF+T2DM patients underwent in vitro NETs release faster than T2DM and HC groups ($P \leq 0.04$) without any stimuli. Under in vitro conditions, serum collected from patients with HF and HF+T2DM induced NETosis in healthy neutrophils significantly higher ($P \leq 0.017$) than serum from HC and T2DM donors. By serum CRP depletion of HF and HF+T2DM, this effect was abolished. We confirmed in vitro that CRP induces a concentration-dependent NETs synthesis.

Conclusion: This study proposes a mechanism by which CRP increases the risk of future cardiovascular events, and supports mounting evidences on the role of neutrophils in chronic low-grade inflammation associated to heart failure.

P087-F | Norbin, a GPCR-adaptor protein and regulator of Prex1, suppresses neutrophil-dependent immunity

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Background: P-Rex family Rac-GEFs are important activators of the small G protein Rac, regulating cell shape, migration, ROS formation, gene expression and cell growth. Neutrophils have a wide range of Rac-dependent responses, including adhesion, chemotaxis, degranulation, phagocytosis and ROS formation. Some neutrophil responses, especially those elicited by GPCR signalling, are Prex1 regulated.

We identified a new regulator of Prex1, the neuronal GPCR adaptor protein Norbin (Pan D.et al, 2016, JBC). This study had revealed that Norbin can bind Prex1, stimulate its Rac-GEF activity and promote its plasma membrane localization. It showed furthermore that Norbin is expressed in neutrophils.

Material and methods: In order to assess the functional importance of the Prex1/Norbin interaction in neutrophils, we generated two new genetically-modified mouse strains: a strain with a conditional Norbin deletion in myeloid cells and a strain with combined Norbin and Prex1 deficiency.

Results: Unexpectedly, we found that isolated Norbin-deficient neutrophils show increased adhesion and spreading,

as well as increased ROS production upon stimulation of GPCRs, and an increased ROS dependent capacity to kill *Staphylococcus aureus* bacteria. Norbin deficiency provides immunity against pulmonary infection with Streptococcus pneumoniae, even in immune-deficient (Prex−/−) mice. Under most conditions, Norbin deficiency overrides the functional impairments caused by the Prex1 deficiency, whereas some effects (e.g. cell spreading) were Prex1-dependent. Mechanistically, the Norbin-deficiency promotes GPCR-dependent Rac1 and Rac2 activity, independently of Prex1. It also increased fMLP-stimulated Erk activity, whereas other GPCR signalling pathways, such as p38Mapk, Jnk and Akt, seemed unaffected, suggesting a degree of pathway specificity.

Conclusion: These data indicate that the GPCR adaptor and Prex1 regulator Norbin plays an important functional role suppressing host defence functions of mouse neutrophils. A subset of Norbin functions are Prex1 dependent, whereas others seem to be regulated through control of GPCR trafficking.

P089-F | Tetrameric S100A8/S100A9 alarmin is a key regulator of cellular dynamics in monocytes

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Phagocytes migrate to sites of inflammation in response to pathogens or tissue damage. Regulatory mechanisms of cell mechanics and dynamics during migration and inflammatory activation of monocytes involve different signaling pathways like changes in intracellular calcium concentration, activation of GTPases and protein kinases. These mechanisms are amplified by endogenous alarmins released during cellular stress or tissue damage. Heterodimeric S100A8/S100A9 is the most abundant alarmin in many inflammatory processes. S100A8/S100A9-dimers are the active form triggering TLR4 dependent pathways whereas their activity is abrogated by calcium-induced tetramer formation.

We used Lifeact ER-Hoxb8 monocytes from wild-type and S100A9 knockout mice to unravel the function of these major calcium binding proteins for cellular dynamics of monocytes. Analyzing S100A9 knockout cells, which are also deficient for S100A8 on the protein level we found major alterations in cytoskeletal dynamics and morphology compared to controls. S100A9 knockout monocytes showed faster basal migration rates, reduced adhesion, lower traction forces and increased activation of GTPases compared to wild-type monocytes. Surprisingly after chemokine activation, only wild-type cells were able to respond with increased migratory activities, whereas S100A9 knockout cells, already pre-activated under basal conditions, remained unaffected. Substitution of extracellular S100A8/A9 tetramers in S100A9 knockout cells reversed all effects leading to a phenotype similar to the resting state of wild-type cells. Interestingly, these effects are mediated by S100-tetramer interaction with CD69 and not via TLR4. Thus our findings demonstrate for the first time an important function of S100-tetramers which shift the inflammatory activity of S100A8/S100A9 dimers to a regulatory one on cellular dynamics of monocytes.

P090-F | Response to DNA by type 1 diabetes patients

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Background: Aberrant recognition of self-nucleic acids by the innate immune system contributes to the pathology of several autoimmune diseases. Even though microbial DNA is recognized mostly by Toll like receptor 9 (TLR9), recent evidence suggest contribution of other cytosolic sequence-nonspecific DNA sensors in DNA recognition. In this study we focused on sensing of microbial and host DNA in type 1 diabetes (T1D) patients.

Methods: Peripheral blood mononuclear cells (PBMCs) and monocytes from paediatric long term treated patients with T1D and healthy donors were stimulated with microbial DNA or neutrophil extracellular traps (NETs). Production of cytokines was measured by Flow Cytometry and multiplex bead assay. Internalization of microbial DNA and colocalization with STING was detected by image cytometry. Furthermore, involvement of TBK1 kinase was investigated, by detecting its phosphorylation with phosphoflow cytometry or by using TBK1 inhibition assay.

Results: We show a prominent proinflammatory response of T1D patient cells, especially monocytes, to microbial DNA in comparison to controls. We further confirm that monocytes bind and internalize DNA and release proinflammatory cytokines. Surprisingly, this production was not affected by TLR9 blockade suggesting an involvement of intracellular receptors in DNA recognition by monocytes.

Furthermore, we detected that TBK1 and STING, two crucial molecules in DNA sensors pathway, are involved in CpG DNA sensing by T1D cells. As a model of host DNA, we used NETs containing self-DNA. In our experimental settings, NET-induced cytokine release was also not abolished after TLR endosome blockade which suggests cytosolic sensing of NET-DNA and was also TBK1 dependent similarly as by microbial DNA.

Conclusions: Here we show significant differences in DNA sensing in a context of T1D patients. We demonstrate that monocytes from T1D patients are able to sense microbes and self- DNA and by signalling engage TBK1 and STING molecules.

P091-F | P84 adaptor subunit reveals to be essential to sustain PI3Kg function in mast cells

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Background: Phosphoinositide 3-kinase gamma (PI3Kγ) is most abundant in cells of hematopoietic origin and it is a key to the regulation of immune cell responses, playing a major role in chronic inflammation and allergy. PI3Kγ is composed of p110γ catalytic subunit and either p84 or p101 adaptor proteins, however in mast cells it operates only as a p84-p110γ hetero-dimer. In mast cells, the complex synergizes with IgE- and antigen- clustered FceRI receptor signaling and it is required to achieve maximal degranulation. During this process, PI3Kγ is activated by ligands of heterotrimeric guanine nucleotide-binding protein (G protein) -coupled receptors.

Methods: Gene-targeted bone marrow-derived mast cells from PI3Kγ, P84 and p101 mice were used to monitor IgE and GPCRs-downstream signaling pathways and its activation, mast cell degranulation, cytokine release and migration.

Results: For the first time using a p84KO mouse model we show in mast cells, that the adenosine/calcium-triggered hyperactivation of the cell is completely abrogated in the absence of the adaptor protein. Furthermore, signaling downstream of FceRI and GPCRs requires p84 presence for Akt/PKB activation. Confirming previous results that the gamma complex plays a major role downstream of the IgE-FceRI signaling pathway. P110γ catalytic subunit alone failed to support IL-6 and TNF-α cytokine release from IgE/antigen- and adenosine-stimulated mast cells. TNF-α downregulation/depletion impairs IgE-induced mast cell recruitment, which links tissue mast cell-derived cytokine release to endothelial activation and mast cell recruitment. P84 was crucial for mast cell chemokinesis, rendering the cells without the adaptor protein significantly unresponsive to IgE/antigen- and GPCRs stimuli.

Conclusions: Our results demonstrate that in a mast cell model, p84 adaptor protein is essential for p84-p110γ complex activation and that, p110γ alone cannot sustain its function. Moreover, we anticipate that in vivo, p84KO animals will have impaired mast cell recruitment leading to protection against passive cutaneous anaphylaxis.

P092-F | Role of mannose-binding lectin in neutrophil infiltration after traumatic brain injury and brain ischemia

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Background: Traumatic Brain Injury (TBI) and stroke are leading causes of death and permanent disability worldwide. Following the primary damage, leukocyte infiltration and the complement cascade are recognized as mechanisms involved in secondary brain damage. It has been reported that pharmacological targeting of mannose-binding lectin (MBL), a protein of the lectin pathway of complement activation, is protective in experimental models of acute brain injury. Also, stroke patients carrying genetic MBL deficiency present smaller infarctions and better clinical outcomes. The aim of this study is to investigate the involvement of the lectin pathway in the immune response mediated by neutrophils in mouse models of TBI and stroke.

Methods: Wild-type (wt) and MBL-deficient mice were subjected to controlled cortical impact as a model of TBI or permanent middle cerebral occlusion (pMCAo) as a model of stroke. Cryostat brain sections were obtained and specific staining for neutrophils was carried out at different time points, i.e. 1 (n = 3), 4 (n = 3) and 15 (n = 3) days after TBI or 1 (n = 3) and 4 (n = 4) days after pMCAo. We studied neutrophil infiltration and regional location by confocal microscopy, and investigated signs of formation of neutrophil extracellular traps (NETs) by assessing histone 3 citrullination and chromatin decondensation. Cell quantification was carried out in different brain regions (cortex, hippocampus and ventricle).

Results: Both TBI and pMCAo caused neutrophil infiltration. We also detected signs of NETosis in brain infiltrating neutrophils of wt and MBL-deficient mice. Compared to wt mice, MBL-deficient mice showed a reduced number of Ly6G+ neutrophils in the hippocampus and ventricle 4 days after TBI.

Conclusion: These preliminary results suggest that MBL participates in secondary neutrophil accumulation in the brain lesion. The putative participation of MBL in neutrophil activation and NET formation is under investigation.

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P093-F | Neutrophil Gas6 protein is involved in neutrophil recognition by microglia for phagocytosis

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Background: Ischemic stroke induces a strong inflammatory response in the brain involving leukocyte infiltration. Microglia, the resident innate immune cells of the central nervous system, phagocytose infiltrating neutrophils[1] [2]. However, the signals involved in neutrophil recognition are not well-known. The objective of this study was to investigate the involvement of the "eat-me" signal Gas6 in the engulfment of neutrophils by microglia.

Methods: We used wild type (wt) mice, Gas6−/− mice, and DsRed reporter mice. We isolated microglia from the adult mouse brain and maintained the cells in culture for 7 days. We then exposed Gas6+/+ or Gas6−/− microglia to Gas6+/+ or Gas6−/− bone marrow-derived neutrophils and studied the cells with multiposition time-lapse microscopy for 10-14 hours. Cultures of Gas6+/+ and Gas6−/− cells were run in parallel in n = 3 independent experiments with 2 replicates per genotype in each experiment. We quantified events by cell tracking with ImageJ.

Results: Compared to Gas6+/+ neutrophils, phagocytosis of Gas6−/− neutrophils by either Gas6+/+ or Gas6−/− microglia was reduced (40%) regardless of microglia genotype (two-way ANOVA by neutrophil genotype and microglia genotype, neutrophil genotype $P < 0.0001$). We also observed signs of neutrophil NETosis, i.e. apparent restructuration of the nucleus, clear expulsion of the intracellular content, increase in cell size, and loss of fluorescence, likely due to enzymatic degradation. We estimated that approximately 10% of neutrophils suffered NETosis. At this point cells looked empty, devoid of cellular organelles and lacked DNA. These structures remained in the culture, but we observed that some of them (15%) were eventually engulfed by microglia.

Conclusion: Microglia recognize and phagocyte neutrophils, including neutrophils that have undergone NETosis. Neutrophil Gas6 expression mediates neutrophil recognition by microglia for phagocytosis.

References: 1. Neumann J et al. (2008) J. Neurosci. 28:5965-75. 2. Otxoa-de-Amezaga et al. (2019) Acta Neuropathol. 137:321-41.

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P095-F | A pathogen and a non-pathogen SFG Rickettsia trigger differential metabolic signatures in macrophage-like cells

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Background: Reductive genome evolution in obligate intracellular Rickettsia has resulted in the loss of many metabolic pathways, which culminates with Rickettsia species being strictly dependent on host cells to survive and proliferate. Several efforts have been made to identify host and bacterial determinants that allow bacteria to proliferate inside host cells. We recently reported a differential tropism of pathogenic and non-pathogenic Rickettsia in macrophage-like cells, further strengthening the complexity of host-rickettsiae interactions and raising questions on how pathogenic Rickettsia manipulate host pathways to their advantage.

Materials and methods: To further understand this, we have herein employed a quantitative high-throughput proteomics approach (SWATH-MS) to profile alterations in THP-1 macrophages infected with R. conorii (pathogenic) and R. montanensis (non-pathogenic).

Results: R. conorii substantially reprograms several host metabolic pathways, modulating host cells to a niche apparently more adapted to its metabolic needs. R. conorii specifically induced the accumulation of several enzymes of the tricarboxylic acid cycle, oxidative phosphorylation, fatty acid beta-oxidation and glutaminolysis, as well as of several inner and outer membrane mitochondrial transporters.

Conclusions: Overall, our proteomic profiling of rickettsiae-macrophage interaction anticipates a profound metabolic rewriting of macrophages by the pathogen R. conorii towards a metabolic signature of an M2-like, anti-inflammatory activation program. This may allow bacteria to obtain the building

blocks necessary for replication and, simultaneously, assure the energetic demands of the host cell. This work adds further knowledge on pathogenicity requirements in rickettsiae and, as excitingly, may help to decipher the still missing ingredients for the design of an axenic culture medium for Rickettsia.

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P096-F | Epigenetic regulation of hibernation: an endogenous switch for safe metabolic suppression?

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Background: Ischemia-reperfusion injury after trauma or transplantation is a field of expertise that is not fully understood yet. Commonly organs are cooled to inefficiently decrease metabolism, leading to high oxidative stress. We are using nature's own way of safe metabolic suppression in a fascinating model: hibernating Syrian hamsters. This process is very rapid and dynamic, which we hypothesize to be regulated by epigenetic mechanisms.

Material and methods: Syrian hamsters were housed at an ambient temperature of 21°C or 5°C to induce hibernation. Animals were sacrificed in different phases of hibernation. Livers were collected and snap frozen.

Global epigenetic changes were detected using LC/MS/MS and immunoblot (histone modifications) and LUMA (DNA methylation).

Whole-Genome Bisulfite Sequencing (WGBS) was performed to identify differentially methylated regions (DMRs). Highly differentially methylated genes were selected as candidate genes for further analysis, e.g. expression measurements (qRT-PCR). Consequently, epigenetic editing was used to modulate expression of these genes in vitro.

Results: Global epigenetic differences were detected for several histone lysines (acetylation of H3, H3K18 and H3K27) throughout the hibernation cycle, whereas global methylation remained stable. Interestingly, we were able to detect differentially methylated regions using WGBS in specific genes, leading to differential expression as measured with qRT-PCR. To mimic safe metabolic suppression in hibernation processes, we used epigenetic editing in vitro. Future experiments will provide insight in downstream effects of these modulations and aid to induce safe metabolic suppression.

Conclusion: During hibernation, epigenetic modifications lead to differential expression of specific genes. These results show a fascinating interplay between epigenetic regulation and metabolism, which could lead to many possible drug targets for safe metabolic suppression. Using epigenetic editing, we aim to mimic hibernation in many clinical applications such as trauma and transplantation.

Funding sources: Graduate School of Medical Sciences (GSMS, University of Groningen) and COST CM1406 supported networking activities (www.epichembio.eu).

P097-F | Physiologic and dietary determinants of intestinal permeability. Presence of a leaky colonic barrier in obese and possible protective effect of Mediterranean diet and moderate extra-virgin olive oil consumption

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Background: Recent studies suggest that intestinal permeability (IP) is essential in maintaining gut-metabolic functions. We aimed to discern the role of age, gender, obesity and diet in modulating IP and “leaky gut”.

Material and methods: Degree of IP was quantified by urinary recovery in 168 adults (M:F = 66:102; age: 45 ± 1.5 years, range: 18-86), triple quadrupole mass-spectrometry and HPLC (AB Analitica, Padua, Italy) of probes with oral sucrose (SO, stomach-duodenum), lactulose (LA) and mannitol (MA, small intestine), and sucralose (SA, colon). BMI classified body size (underweight-obesity). Dietary habits and Mediterranean Diet adherence (MDA, score 0-18) were registered. Liver steatosis was assessed by ultrasonography (Noblus Hitachi, Japan; grade 0-3).

Results: IP was comparable in both sexes. Gastric and IP were similar according to age, BMI and degree of steatosis. IP increased with BMI and degree of steatosis ($P < 0.001$), correlated inversely with age and was higher in obese (1.41 ± 0.14%) than in overweight (1.14 ± 0.07), lean (1.05 ± 0.04%) and underweight subjects (0.9 ± 0.1%,

$P = 0.008$). Those with "sufficiently adequate" MDA (score 10-15) had lower IP (1.09 ± 0.1) than those with "scarcely adequate" MDA (score 5-9, $1.3 \pm 0.1\%$, $P = 0.04$). IP was independent of consumption of fruit, potatoes, dairy products, nuts, legumes, fish, and white/red meat. Subjects consuming extra-virgin olive oil (EVO) 1-2 times/day had lower IP and degree of steatosis than those consuming EVO 3-4 times/day ($1.11 \pm 0.5\%$ vs $1.63 \pm 0.8\%$, $P = 0.005$; $0.5 \pm 0.8\%$ vs $1.6 \pm 0.8\%$, $P < 0.001$; respectively).

Conclusions: Colonic permeability decreases with age and increases with BMI. Obese subjects display a leaky colonic barrier with higher permeability at higher steatosis. IP is unrelated to fruit, potatoes, dairy products, nuts, legumes, fish, and white/red meat consumption. Further studies will confirm if MDA and moderate EVO consumption bring beneficial effects on colonic permeability, liver steatosis and obesity.

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P098-F | Serum levels of osteopontin predict diabetes remission after bariatric surgery

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Background: Bariatric surgery was shown to effectively improve glycemic control in morbid obese subjects. However, the molecular basis of this association are still elusive and can act independently of weight loss. Here, we retrospectively investigated the inflammatory molecule osteopontin (OPN), as a potential predictor of type 2 diabetes mellitus (T2DM) remission.

Material and methods: Baseline serum levels of OPN were analyzed in 41 diabetic patients who underwent bariatric surgery. Anthropometric measures, biochemical variables, including insulin sensitivity (HOMA2 indexes), were assessed at baseline, 1 and 3 years after surgery.

Results: At baseline, patients that experienced T2DM remission had increased waist circumference, body weight and BMI and higher serum OPN as compared to non-remittent ones (Figure 1). Both patients with T2DM remission and non-remission improved lipid and glucose profiles. Insulin resistance indexes were only improved in the T2DM remission group (Figure 2). In the overall cohort including both T2DM remission and non-remittent patients, baseline circulating levels of OPN significantly correlated with over-time reduction of body weight and BMI, and improved insulin sensitivity as well. However, only HOMA2-%S remained independently associated with serum OPN in multivariate linear regression analysis (B 0.227 [95% CI 0.067-0.387]; β=0.831; $P = 0.010$) (Figure 3). Baseline values of OPN predicted 3-year T2DM remission independently of body weight loss, improvement in BMI and duration of diabetes (OR 1.046 (95% CI 1.004-1.090); $P = 0.033$) (Figure 4).

Conclusion: Although larger studies are needed to confirm our preliminary results, pre-operative OPN serum levels might be useful to predict 3-year T2DM remission independently of weight loss in patients treated with bariatric surgery.

P100-F | The protective effect of walnuts as dietary fat source on the functionality of liver and adipose tissue

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Background: Consumption of Western-type diet is related to an increasing prevalence of cardiovascular and metabolic diseases. Therefore, there is a growing interest towards functional foods, such as walnuts. The aim of the project was to evaluate the effect of dietary walnuts on liver and adipose tissue morphology and functionality in an apoE-knockout (apoE-/-) mice model of Western diet-induced atherosclerosis.

Material and methods: Forty-five male apoE−/− were fed three experimental diets (n = 15/group): a regular rodent CHOW (10% of fat), a High Fat Diet (45% of energy as fat), or walnut-enriched High Fat Diet (W-HFD), for 15 weeks. Food intake and body weight were recorded weekly. At the end of the experimental period liver, visceral (VAT), subcutaneous (SAT) and brown (BAT) adipose tissues were analysed.. Evaluation of Non Alcoholic Fatty Liver Disease (NAFLD) and hypertrophy of adipose tissue was performed in Hematoxylin-Eosin stained

paraffin sections. Gene expression in adipose tissues was determined by RT-PCR.

Results: No differences in food intake were observed. Body weight of the animals fed W-HFD was greater than CHOW ($P > 0.05$). In liver, both HFDs induced micro and macrovesicular changes and inflammation vs CHOW diet ($P > 0.05$). However, mice on W-HFD had milder alterations than those on HFD, although not significantly. In adipose tissue lower hypertrophia and "whitening" of BAT were observed in W-HFD group. Hypertrophy of SAT in these animals was greater than the rest of the groups (all, $P > 0.05$). Finally, the expression of the tested genes was differentially affected by the inclusion of walnuts in the diet.

Conclusions: These results indicate that walnuts might protect adipose tissue from the pathological changes promoted by a western type diet, improving the functionality of this organ. These data reinforce existing evidence that identify walnuts as a potentially cost-effective strategy to ameliorate metabolic diseases associated with inadequate dietary patterns.

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P101-F | Helicobacter pylori infection from diagnosis to treatment. A "real-life" scenario in Apulia, Southern Italy

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Background: Helicobacter pylori (HP) chronic gastritis is the most common bacterial infection worldwide, which may cause peptic ulcer disease, gastric metaplasia, atrophic gastritis, gastric cancer, and mucosa-associated MALT lymphoma. In a "real-life" scenario in Italy, we assessed the association between HP diagnosis (13-C Urea Breath-Test, UBT), smoking habits, familial history of HP infection, and distinct therapeutic regimens.

Material and methods: In 2018, 632 subjects (M:F = 242:390, 51 ± 18.5 years) with suspected HP infection underwent UBT (Richen Europe Srl, Milan, Italy; 75 mg 13C urea) in a 3rd referral center in Southern Italy.

Results: Naïve patients were 62% (n = 392), while 38% (n = 240) underwent UBT re-evaluation after eradication therapy. Smokers were 112 (17.7%). HP(+)ve subjects were comparable (P = NS) between smokers (26/112, 23%) and non-smokers (122/520, 23%). Familial HP history was positive in 228 subjects (36.1%). Prevalence of HP(+)ve subjects was significantly greater when familial history was positive compared to subjects with negative familial history (100/228, 43.9% vs 128/404, 31.7%; $P = 0.0022$). In the 240 patients requiring therapy, eradication rates [%] were greatly influenced by prescription scenarios (i.e., family medicine or referral center). Adopted regimens (1st, 2nd line) were: 14-days concomitant PPI+claritro+amoxi [98.3%]; quadruple 'Pylera' PPI+Bismuth sc+metronidazole+tetracycline [93.8%]; 10-days sequential PPI+claritro+amoxi [88%]; 7-days triple PPI+claritro+amoxi [63%]; 10-days PPI+Levofloxacin+amoxy" (57.7%). Notably, 62% of treated patients were unaware about the exact eradication regimen (likely triple therapy, eradication in 64%, given elsewhere).

Conclusions: In a referral center in Southern Italy, we observe that HP infection rate is slightly higher in subjects with a positive familial history. Still, UBT is overprescribed (60-70%), even in the presence of poorly HP-related symptoms or even in asymptomatic subjects. Although noninvasive, unnecessary tests lead to long waiting lists, and increased expenditures. Highly discrepant therapeutic regimens cause unsatisfactorily eradication rates, need for re-treatments, alarmism among patients, and potential dangerous antibiotic exposure.

P102-F | Bromocriptine decreases hepatic steatosis by modulation of the dopamine signaling in liver

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Background: The D2 agonist Bromocriptine was approved by the FDA to improve insulin sensitivity and Type 2 Diabetes and may have beneficial effects in associated pathologies like non-alcoholic fatty liver disease (NAFLD). Although the underlying mechanisms remains unclear, Bromocriptine may have a direct effect on various insulin-sensitive organs. This study evaluated the improvement of insulin sensitivity and reversion of hepatic steatosis in an animal model of obese type 2 diabetes.

Material and methods: Wistar (W) rats fed a normal diet and non-obese type 2 diabetic Goto-Kakizaki (GK) rats were divided into 4 groups: GK with normal diet, GK with obesity induced by a high fat and sucrose diet, GK obese treated with Bromocriptine 10 mg/kg/d for 30 days and obese GK treated with vehicle. The glycemic and lipid profiles, insulin tolerance and insulin and dopamine signaling in liver were evaluated at fasting and 1 hours after a mixed diet ingestion. In addition, hematoxylin-eosin staining of liver was performed.
Results: Rats maintained on a fat diet revealed a worsening of fasting glycemia, cholesterol and triglycerides, as well as insulin tolerance, which were reverted with the administration of Bromocriptine. In the liver, there was an evident reduction of hepatic steatosis and increase of GLUT2 levels in Bromocriptine-treated rats, suggesting an improvement of glucose uptake and fatty acid metabolism, but no changes in insulin signaling were observed. However, there was a postprandial increase in dopamine D1 receptor levels and a decrease of fasting and postprandial D2 levels.
Conclusions: Our results suggest that Bromocriptine acts directly in the liver modulating dopamine signaling, which is associated with an increase of GLUT2-mediated glucose uptake and a reduction of hepatic steatosis. Although further studies are still required, such results suggest that Bromocriptine may be effective in reducing hepatic lipotoxicity, which may have a positive impact on insulin sensitivity.

P103-F | Treatment with osteocalcin, an osteoblast-secreted hormone, ameliorates insulin resistance and adipose tissue function in a lean model of T2D

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Background: Primarily regarded as a dynamic scaffold, increasing evidence demonstrates that bone is an endocrine organ that actively regulates metabolism. Osteoblast-secreted osteocalcin on its uncarboxylated form (uOCN) stimulates insulin secretion by the pancreas and enhances insulin sensitivity in peripheral tissues. Thus, reduced uOCN levels are associated to dysfunctional glucose metabolism and insulin resistance, linking bone to type 2 diabetes (T2D). Our preliminary observations in Goto-Kakizaki (GK) rats, a lean model of spontaneous T2D, shown reduced uOCN levels compared to healthy animals. Therefore, this study aimed to evaluate uOCN effects on insulin sensitivity, and ultimately on the metabolic outcome of T2D, in both GK rats and their control strain (Wistar-Han).
Material and methods: During 7 weeks, 12-week old male diabetic and control animals were daily injected either uOCN or vehicle (n = 10 animals/group), while their blood glucose levels, body weight, and food intake were weekly monitored. At the end of the treatment, intraperitoneal glucose tolerance and insulin sensitivity tests were performed, and the area under the curve (AUC) determined; blood samples and tissues were collected for analyses.
Results: From 3-weeks of treatment, T2D animals receiving uOCN had a 30% reduction in blood glucose levels compared to vehicle ($P = 0.03$), while healthy animals showed no effects of the treatment. Neither body weight nor food intake were altered by uOCN administration, but treated T2D rats had lower amount of visceral adipose tissue ($P = 0.02$). Their leptin-to-adiponectin ratio, a marker of adipose tissue dysfunction and insulin resistance, was also reduced ($P = 0.002$). Improved insulin sensitivity in T2D treated group was further verified by AUC decrease on insulin sensitivity tests ($P = 0.02$) and on HOMA-IR, namely due to the reduction of plasmatic insulin levels.
Conclusion: Our results support the importance of uOCN as an insulin-sensitizing hormone, and ultimately indicate the potential of bone as a therapeutic target in T2D.

P104-F | Fructose contributes more to the synthesis of saturated fatty acids than it does to oleate in the liver

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Background and aim: A high intake of fructose is strongly associated with obesity and hepatic lipotoxicity. Lipotoxicity is related to both the amount and type of fatty acid, with saturated fatty acids (SFA) being considered to be more lipotoxic than mono- or polyunsaturated fatty acids (MUFA, PUFA). Since both SFA and MUFA can be synthesized via de novo lipogenesis (DNL), our objective was to compare the contribution of exogenous fructose, in the presence of an equal amount of glucose, to the synthesis of SFA and oleate - the principal MUFA species of hepatic triglyceride (TG). This was achieved by 13C NMR isotopomer analysis of hepatic TG of mice that had ingested 13C-glucose and 13C-fructose.
Material and methods: At the start of the dark period in a 12/12 hours dark/light cycle, 9 C57/BL6 mice (4M, 5F) fed with standard chow were given an i.p. injection of 99.9% 2H2O/0.9% NaCl to raise body water 2H-enrichment to ~4%.

The drinking water was supplemented with 17.5% w/w unlabeled glucose and 17.5% w/w fructose enriched to 20% with [U-13C]fructose. Animals were allowed to feed naturally overnight and then sacrificed at the end of the dark cycle. Livers were freeze-clamped and triglycerides were extracted and purified from other lipid species and analyzed by 13C NMR.

Results: During overnight feeding, 4.6 ± 0.4% of TG-SFA was accounted by DNL from [U-13C]fructose compared to only 2.8 ± 0.3% of TG-oleate ($P = 0.021$ vs SFA).

Conclusion: DNL from fructose contributes more to the appearance of SFA than it does to oleate. Hence, in addition to contributing additional hepatic fatty acids, it modifies the overall fatty acid distribution towards a more lipotoxic profile.

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P105-F | Transfer of 2H from glucose to fatty-acids during de novo lipogenesis in feeding mice

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Background and aim: Deuterated water (2H2O) is used for quantifying fatty-acid synthesis. A major uncertainty of this method is that exchange of water hydrogens with those of fatty acid precursors is incomplete. By monitoring transfer of 2H from glucose into fatty-acid position-2 (2H originating via malonyl-CoA), position-3 (2H originating via NADPH), and methyl (2H originating via acetyl-CoA), the fraction of fatty acid hydrogens derived from glucose (i.e. had not undergone exchange with water) was estimated.

Material and methods: Nine adult male C57BL/6 mice fed with standard chow were given overnight access to drinking water containing 15 g/100 mL fructose and 15 g/100 mL glucose enriched to 20% with [U-2H7]- and [U-13C6]glucose. In the morning, liver triglycerides were isolated, purified and subsequently analyzed by 13C and 2H NMR spectroscopy. The 2H/13C-enrichment ratios in position-2, position-3 and methyl of triglyceride fatty-acids was used to determine the fraction of glucose hydrogens transferred into each site.

Results: The fraction of glucose 2H transferred into fatty-acid position-2, position-3 and methyl was 7 ± 2%, 23 ± 6% and 11 ± 3%, respectively. Extrapolating these results to the entire 31 hydrogens of palmitate indicates that only 23 ± 2 out of 31 were derived from body water.

Conclusion: During lipogenesis, glucose 2H are transferred at different fractional rates into different fatty-acid sites. This information allows better modelling of lipid enrichment from 2H2O and also provides insight on NADPH sources for lipogenesis.

P106-F | Impact of added and naturally present sugars of fruit juices on glycemic index and glycation

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Background: High sugars consumption is related to the development of chronic metabolic diseases, being beverages one of sugars' major dietary source. Nevertheless, little is still known about the different impact of naturally present fruit sugars and added sugars in beverages on oxidative stress and advanced glycation end-products. In this project our objective is to assess the different impact of commercial fruit juices only with natural sugars with the same amount of added sugars in a first step on the glycemic index and complementary on oxidative and glycation markers.

Methods: After a 6 hours fasting, 12-week old Wistar rats were fed with 2, 3 or 4 mL of each juice or solution with the same profile of sugars by gavage. Glycemia was measured in the tail vein before and 15, 30, 45 and 60 minutes after gavage.

Results: Wistar rats were fed with different juices' volumes, looking for a glycemic index peak, which was obtained for 4 mL. This is equivalent to around 3% of dairy caloric ingestion (red fruit, 3.17%; peach, 2.98%; orange, 2.86%; pear, 3.23%) and 60 Kcal in humans (red fruit, 63.34Kcal; peach, 59.63KCal; orange, 57.19KCal; pear, 64.6KCal), being equivalent to the consumption of around 125 mL of juice. Then juice consumption was compared with a sucrose, glucose and fructose solution with the same sugar's profile of each juice. Each of the fruit juices had a lower glycemic index than the solution with added sugars, being the peach juice the one with the bigger difference.

Conclusions: Such results demonstrate that added sugar increase more the glycemic index than natural fruit sugars and also that the fruit's composition has an action in the glycemic index. Further impact will be demonstrated through the evaluation of post-prandial levels of oxidative stress markers and glycation.

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P107-F | Modulation of dopaminergic signaling with Bromocriptine improves glucose and lipid metabolism in white and brown adipose tissue of an obese type 2 diabetic animal model

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Background: Dopamine is an important neurotransmitter which actions are mediated through D1R and D2R receptor family's activation. Its signaling machinery has been identified in adipose tissue, suggesting a role in carbohydrate and lipid metabolism regulation. Therefore, the modulation of dopamine could constitute an important target for the treatment of obesity and type 2 diabetes. Bromocriptine is a D2 dopamine receptor agonist approved by the Food and Drug Administration for the treatment of type 2 diabetes since it had shown positive impact on glycemic control and insulin sensitivity. However, its peripheral actions on insulin sensitive tissues remain unclear. Our main goal is to evaluate whether the modulation of peripheral dopaminergic signaling improves glucose and fatty acid metabolism in white and brown adipose tissues.

Methods: Obese type 2 diabetic Goto-Kakizaki rats were treated with Bromocriptine for 4 weeks (10 mg/kg/d) and the mechanisms of glucose and fatty acid metabolism have been assessed after an overnight fasting and post-prandially (1 hours after a mixed meal ingestion).

Results: Bromocriptine treatment significantly improved glucose and lipid metabolic markers while increasing insulin tolerance. Increased D1R expression after fasting in both tissues was also observed after Bromocriptine treatment, which wasn't observed pos-prandially. The activation of AMPK was differentially induced in both tissues, being higher in white adipose tissue after fasting. Moreover, GLUT4 expression were increased, after fasting and sustained post-prandially mostly in brown adipose tissue.

Conclusions: Bromocriptine treatment improves glucose uptake and fasting lipid metabolism due to increased fatty acid catabolism. These mechanisms are associated with improved insulin sensitivity in white and brown adipose tissue, possibly through different regulation of dopaminergic signaling through D1R during fasting and postprandial periods, which leads to decreased lipotoxicity.

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P108-F | Perivascular adipose tissue in obesity: friend or foe

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Background: Obesity is a growing problem worldwide often associated with increased risk of premature death, morbidity, and mortality. Perivascular adipose tissue (PVAT), the adipose tissue that surrounds most of the vasculature, has emerged as an active component of the blood vessel wall regulating vascular homeostasis. PVAT exerts an anticontractile effect in response to various vasoconstrictor agonists, and this is lost in obesity. The main goal of this study was to investigate the effects of high-fat diet on PVAT and study its impact on endothelial function of mesenteric arteries of obese animal models.

Materials and methods: Four-month-old male Wistar (W) rats were randomly divided in two subgroups: group 1) W control group fed with standard diet (W8 m); group 2) W rats fed with high fat diet for 4 months (WHF). Glucose, lipids and adipokine concentrations were measured on blood samples. Contractility studies were performed using wire myography on mesenteric arteries with and without PVAT and functional endothelial-dependent and independent vasorelaxation was evaluated in the different groups. Oxidative stress and inflammatory biomarkers were also evaluated in arteries and PVAT of the different groups of rats. Changes in the PVAT environment were assessed using immunofluorescent.

Results: High fat diet induced significantly increased body weight, glucose at 2 hours and systemic levels of free fatty acids, leptin and leptin/adiponectin ratio. It also significantly reduced the efficacy of NO-dependent vasorelaxation in mesenteric arteries accompanied by 2-fold increment in vascular oxidative stress. In WHF group, PVAT significantly increased the expression of pro-inflammatory chemokines (i.e monocyte chemotactic protein-1/CCL2, RANTES/CCL5) and adipokines (i.e leptin) compared to control W rats, probably important contributors of endothelial dysfunction.

Conclusions: Inflammation in PVAT directly impacts vascular disease of the underlying artery, perhaps contributing to the endothelial dysfunction underlying atherosclerosis.
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P109-F | The effects of second-generation antipsychotics on human subcutaneous adipose tissue metabolism

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Background and aims: Schizophrenia is a severe mental condition affecting around 21 million people worldwide reducing their life expectancy by 10-20 years due to high prevalence of metabolic syndrome (MetS) and cardiovascular diseases. Second generation antipsychotics (SGAs) induce MetS in 35-48% patients, further increasing their risk of cardiovascular complications. Individual SGAs induce MetS to a different extent, e.g., while Olanzapine is a high-risk drug, Aripiprazole is a metabolically neutral medication. The mechanisms underlying such side-effects are not fully elucidated. The aim of our study was to investigate the direct effect of SGAs on human subcutaneous adipose tissue metabolism as well as adipose tissue inflammation, as a substantial component of MetS.
Materials and methods: Subcutaneous adipose tissue needle biopsies were obtained from 46 healthy SGA-free volunteers (13M, 33F; age: 20-76 years; BMI: 20.9-35.0 kg/m^2). Isolated adipocytes were pre-incubated without (control) or with olanzapine (0.004-20 μmol/L) or aripiprazole (0.02-100 μmol/L) for 30 min at 37°C. Then, basal and insulin-stimulated D-[U-14C]-glucose uptake (n = 15) and basal and isoproterenol-induced lipolysis (n = 6, w/wo 0.5 μmol/L isoproterenol and 0.1-100 mU/mL insulin) were measured. Same experiments and gene expression analysis were conducted on adipose tissue that was incubated with mentioned concentrations of both drugs for 24 hours (n = 13) and 72 hours (n = 12). The inflammatory gene expression measurements are in progress.
Results: Both short- and long-term incubation of adipocytes and adipose tissue with therapeutic concentrations of the drugs did not change basal or insulin-stimulated glucose uptake. Both drugs did not have an effect on the basal and isoproterenol-stimulated lipolysis after short-term incubation.
Conclusion: Our data show that SGAs do not seem to have a direct effect on human subcutaneous adipose tissue glucose uptake and lipolysis. There is a need to study the impact of SGAs on inflammation induction in adipose tissue and these experiments are in progress.
Funding source: ITN TREATMENT.

P110-F | Comparison GheOP3S tool and START/STOPP criteria for screening of potentially inappropriate medications and omissions in nursing home residents

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Background: There is limited information about the comparative effectiveness of the START/STOPP criteria and the GheOP3S tool, for the screening of potentially inappropriate prescription (PIP) in the geriatric population. Considering this, the aim of this study was to compare the ability of START/STOPP criteria and GheOP3S tool to identify the potentially inappropriate prescribing (PIP) and potential prescribing omissions (PPO).
Methods: This is a retrospective observational study where a total of 422 subjects were included. The Charlson Comorbidity Index (CCI) and the Medicines Co-morbidity Index for older people (MCI) were used to determine the comorbidity status. The user's diagnosis and medications prescribed were analyzed with START/STOPP criteria and GheOP3S tool. The Wilcoxon signed rank test was used to compare these criteria. The statistical relationship between the occurrence of PIP and users age, the number of medication prescribed, the number of diagnoses, CCI, and MCI was determined with one-tailed bivariate correlation.
Results: START/STOPP criteria detected 843 PIPs and 1067 PPOs, while GheOP3S tool detected 936 PIPs and 202 PPOs. The GheOP3S tool detected significantly more PIPs than STOPP criteria ($P = 0.003$). A significantly higher number of PPOs were detected with START criterion ($P < 0.0001$). The results obtained with START/STOP criteria positively correlated with mentioned variables. Oppositely, there is a negative correlation between the results obtained with GheOP3S tool and age. Still, the positive correlation could be found with the rest of the variables.
Conclusion: The results of this study indicate that both tested tools demonstrated efficiency to detect PIPs and PPOs, The GheOP3S tool detected significantly more PIPs than STOPP criteria. On the other hand, START criteria performed much better for the screening of PPOs.

P111-F | First-line therapy for H. pylori infection with sequential therapy: comparison between 10- and 14-day regimen

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Background: Standard 10-day sequential therapy is advised as first-line therapy for H. pylori eradication by current Italian guidelines. Some data suggested that a 14 days regimen may achieve higher eradication rates. This study compared the efficacy of sequential therapy administered for either 10- or 14 days.

Methods: This prospective, multicenter, open-label study enrolled patients with H. pylori infection, never previously treated. Patients were receiving a sequential therapy for either 10 or 14 days with esomeprazole 40 mg and amoxicillin 1 g (5 or 7 days) followed by esomeprazole 40, clarithromycin 500 mg and tinidazole 500 mg (5 or 7 days), all given twice daily. Bacterial eradication was checked by using 13C-urea breath. Cure rates were calculated at both Intention-to-treat (ITT) and per-protocol (PP) analyses.

Results: A total of 291 patients were enrolled, including 146 patients in 10-day and 145 in the 14-day regimen. The eradication rates were 87% (95% CI = 81.5-92.4) and 90.3% (95% CI = 85.5-95.1) at ITT analysis with the 10- and 14-day regimen, respectively, and 92.7% (95% CI = 88.3-97) and 97% (95% CI = 94.2-99.9) at PP analysis (P = NS). Among patients who earlier interrupted therapy, bacterial eradication was achieved in 8 out of 9 who completed the first therapy phase and performed at least ≥ 3 days of triple therapy in the second phase.

Conclusions: This study found that both 10- and 14-day sequential therapies achieved high eradication rate for first-line H. pylori therapy in clinical practice.

P113-F | Return to work after ischemic stroke treated with thrombectomy

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Background: Using endovascular thrombectomy (EVT) as a therapeutic in the acute phase of an ischemic stroke is associated with a better functional outcome. Nonetheless, the impact of EVT in returning to work after a stroke is still unknown. We aimed to define the influence of EVT on employment after large vessel occlusion (LVO) stroke.

Material and methods: Case-control study including all patients with an LVO admitted in the tertiary hospital between 2015 and 2017, age <70 years, paid job until onset of symptoms. Back to work and functional state were assessed in person or by telephone. We considered as professionally active having a remunerated job; as functional independence a modified Rankin scale(mRS) between 0-2 and as absence of disability an mRS between 0-1. We performed a descriptive statistic of the functional and working state of all patients, through a binary logistic regression adjusting for confounders and group differences. Significance value was set at $P < 0.05$.

Results: 101 patients fulfilling the inclusion criteria were submitted to thrombectomy. Mean age 54.30 ± 10.76 years, 56.4% men, mean NIHSS at admission 16.88 ± 7.76. A year after stroke 57 (56.4%) were functionally independent, 44 (43.6%) didn't have disabilities and 52 (51.5%) had a job. The employment univariate analysis was associated with low mRS (1.14 ± 0.16 vs 1.91 ± 0.27 $P < 0.001$) and younger age (51.06 ± 10.75 vs 57.73 ± 9.75 $P = 0.02$). 75 patients not submitted to thrombectomy were part of the control group. Mean age 59.57 ± 9.56 years, 53% men, mean NIHSS at admission 13.85 ± 7.32. One year post-stroke the control group was less employed (51.5% vs 28.0%, $P = 0.002$). Thrombectomy was associated with a greater paid job rate after adjusting for confounders (OR: 4.07; IC 95%: 1.84-8.99; $P = 0.001$).

Conclusion: EVT is linked to a significant higher rate on returning to work 1 year post LVO stroke. This therefore reinforces its strong social impact.

P114-F | Ameliorated antibacterial photodynamic therapy in purulent-inflammatory diseases of soft tissue treatment

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Here we present the results of the complex treatment of purulent-inflammatory diseases of soft tissues using the ameliorated technique of photodynamic antimicrobial therapy (patent #181767). Two groups of patients with the purulent wounds of the upper/lower extremities underwent the complex treatment of purulent-inflammatory diseases by the combination of active surgical tactics followed by improved photodynamic antimicrobial therapy (1 group), and in group 2, the classical tactic was used with the postoperative usage of the antibiotical therapy and local treatment with gel bandages. The coherent light source used for photodynamic antimicrobial therapy was updated by the added XY scan option that allowed us to cover larger territories by the light irradiation without a significant loss of its power. As the photosensitizer, we have used toluidine blue because of its low price. The results of our study demonstrated that the recovery period in the first group was strongly reduced. The postoperative wound was cleaned out the pus more rapidly (3.2 ± 0.4 days vs 4.3 ± 5 days in the second group), the duration of perifocal edema inflammation (6.4 ± 0.7 days vs 8.8 ± 1.3 days in the second group) and wound healing was strongly reduced (13.5 ± 0.8 days vs 17.3 ± 1.5 days in the second group). We have equally found that while in 34.26% of cases the sensitivity of seeded microorganisms to antibiotics showed high antimicrobial resistance to widely used in purulent diseases antibiotics (oxacillin, ceftriaxone, clindamycin, lincomycin), the antibacterial photodynamic therapy showed to be highly sensitive to all kinds of microorganisms involved in the development of purulent infection.

Altogether, we suggest that ameliorated antibacterial photodynamic therapy could be efficiently included in complex therapy of the purulent disease treatment, associated with contamination of the bacteria resistant to widely used antibiotics.

P115-F | Efficacy of pharmacotherapy in post-stroke shoulder pain

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Background: Shoulder pain occurs in 70% of patients after cerebral stroke. Post-stroke shoulder pain (PSSP) has an unfavorable prognosis, significantly reduces the effectiveness of rehabilitation and the quality of life. There are currently no unified guidelines for PSSP pharmacotherapy.

Material and methods: 210 patients with PSSP (137 men and 107 women) with stroke duration from 1 month to 2 years were examined, average age was 58.6 ± 10.53 years. The intensity of pain was assessed by VAS in mm. The treatment included successively: lidocaine intravenously, 80 mg in 100 mL of isotonic sodium chloride solution (10 infusions), non-steroidal anti-inflammatory drug for 7 days, tizanidine 6 mg per day 7 days, amitriptyline 25-75 mg per day. Other treatment at the time of the study was canceled.

Results: Pain intensity according to VAS before treatment was 64.2 [35; 95] mm, after applying lidocaine - 54.3 [25;85] mm, after non-steroidal anti-inflammatory drugs - 42.9 [0; 80] mm, after amitriptyline - 31.7 [0;70] mm. After completion of treatment, the pain score according to VAS was 31.7 [0;68] ($P < 0.00001$ when compared with results before treatment). The average decrease of pain after lidocaine infusions was 16.9 [0;40]%, after nonsteroidal anti-inflammatory drugs - 20.9 [0; 57.1]%, after centrally acting drugs - 19.4 [3; 66.7]%. Monotherapy with any drug did not have significant differences in comparison with the norm, only the results of the full scheme of treatment had statistically significant differences from those before the start.

Conclusions: The results of our study showed that in central sensitization, tizanidine and amitriptyline are most effective. In peripheral sensitization, simultaneous administration of nonsteroidal anti-inflammatory drugs and centrally acting drugs (tizanidine or amitriptyline) is recommended.

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P117-F | Characterization of sperm function in patients with idiopathic infertility

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Background: Infertility is a worldwide growing problem, to which the male factor is an important contributor. There are several known causes of male infertility, however in about half of the cases a cause cannot be determined - idiopathic male infertility (IMI). The male infertility diagnosis is mainly based on routine seminal analysis; however, this analysis has a limited predictive power either regarding sperm function or in terms of fertilization success. With that in mind, the purpose of this work is to understand the mechanisms that underlie

IMI, through a detailed characterization of sperm function, being the ultimate goal to find a biomarker for these patients.

Material and methods: Human normozoospermic (Control group) and idiopathic sperm samples were obtained after density gradient centrifugation and evaluated microscopically in terms of motility, viability (Eosin Y), morphology and nuclear chromatin status (Diff Quik Stain), according to the World Health Organization guidelines. The capacitation status (Phosphotyrosines) and the acrosome integrity (PSA-FITC) were assessed immunocytochemically and by fluorescence, respectively.

Results: Viability, motility and normal morphology presented higher percentages in the control group. The integrity of the acrosome and the % of capacitated sperm were also negatively affected in the idiopathic group. Conversely, the status of the nuclear chromatin seems to be similar in both control and idiopathic group.

Conclusions: Besides the viability, motility and morphology, also sperm capacitation and acrosome status seem to be affected in the idiopathic group. The results regarding the nuclear chromatin status suggest that IMI is not associated with sperm chromatin alterations. However, the relevance of each of these altered parameters to the infertility condition of our patients and which one contributes more significantly to it, is still to uncover.

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P119-F | Genetic variability of influx and efflux transporters: the impact on susceptibility to chronic myeloid leukemia development

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Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by the presence of BCR-ABL fusion gene. Beside the clinical success of the treatment with tyrosine kinase inhibitors (TKIs), resistance to TKIs had stimulated the research of the mechanisms involved, including changes on influx and efflux transporters, that affect intracellular drug concentrations. Influx (OCT1, OCTN2) and efflux transporters (PgP, BCRP) are responsible for the intrusion or extrusion of substrates, respectively, such as nutrients, metabolic products, xenobiotics and anticarcinogenic drugs. The genetic variants that could affect the function of these transporters may influence cancer risk. In this study we investigate the influence of polymorphisms in genes related with drug transport genes (ABCB1, ABCG2, SLC22A1 and SLC22A5) on CML susceptibility.

Ten genetic variants in ABCB1, ABCG2, SLC22A1 and SLC22A5 were genotyped by tetra-primers-AMRS-PCR in 198 patients with CML and 404 controls. The role of these genes polymorphisms in CML susceptibility was assessed by logistic regression analysis and/or by Fisher's exact test, with $P < 0.05$ considered as significant.

The results showed that individuals with AA genotype (rs1045642 ABCB1) are at higher risk of developing CML [Odds ratio (OR): 1.92; 95% confidence interval (CI): 1.134-3.281; $P = 0.015$] while GG genotype is a protective factor [OR: 0.622; 95% CI: 0.438-0.884; $P = 0.008$]. The GG genotype of ABCG2 gene (rs2231142) was also associated with high risk of CML development, while allele T present a protective action [OR: 0.589; 95%CI: 0.388-0.892; $P = 0.001$]. In both SLC22A5 SNPs we observed that the recessive allele (G for rs274558 and C for rs2631365) confers a protection, where individual with at least one recessive allele present lower risk to CML development. We also observed an association between different haplotypes and the genotypic profile and CML development.

In conclusion, our results suggest that genetic polymorphisms in influx and efflux transporters genes might be risk factors for CML development.

P120-F | Progesterone treatment implication in immunohistochemical markers shift in endometriosis implants

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Background: Endometriosis represents a common gynecological pathology, described as an inflammatory,

estrogen-dependent disease defined by endometrial stroma and glands outside the uterus. The etiology is genetic and immune while hormonal and environmental factors are usual triggers.

Material and methods: According to immunohistochemistry (IHC)assessment, hormonal receptors - estrogen (ER) and progesterone (PR) have significantly altered expression in endometriosis implants when compared to normal endometrium. We underwent a prospective study which included 23 patients aged 22 to 39 diagnosed with endometriosis. 9 patients received progesterone treatment with 0.075 mg desogestrel 24 weeks preoperative and 14 patients were operated without treatment. The study aimed to highlight the modifications involving the expression of ER, PR, B-cell lymphoma 2 (Bcl-2) and Ki-67 markers in the endometriosis implants after progesterone therapy.

Results: In our study administration of oral desogestrel proved beneficial in the management of endometriomas by inducing molecular changes in the expression of different markers, especially increasing the expression of PR and decreasing Ki-67 expression. Clinically it caudes an improvement of symptomatology, a decrease in endometrioma volume and of the intraoperative circumstances. The PR expression was significantly increased in the stroma, compared to those without treatment ($P = 0.025$). This result suggests an increase of progesterone stromal sensitivity in treated patients, while in the same group we recorded a decrease in epithelial PR expression. Our research suggests that oral progesterone has a limited effect on the ER expression both in the stroma and the epithelium. The Bcl-2 expression is controlled by progesterone, considering that after treatment, it increased exponentially especially in the stroma but also in the epithelium.

Conclusion: Our study underlines the importance of progesterone therapy on the expression of hormonal receptors on endometriosis implants, but also on Bcl-2 and Ki-67 markers of cell apoptosis and proliferation.

P121-F | Therapeutic drug monitoring of levetiracetam: pharmacokinetic characterization and compliance assessment of epileptic patients

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Levetiracetam is a second-generation antiepileptic drug (AED) widely used in the treatment of focal and generalized epilepsy, particularly due to its pharmacokinetic and safety profiles. Its pharmacokinetic monitoring is ascribed as useful to personalize the dosing regimens of levetiracetam and to assess drug compliance.

This study aimed at evaluating patient compliance to levetiracetam and characterizing the pharmacokinetics of this AED for the first time in a Portuguese population of epileptic patients.

Thereby, a retrospective study was performed from January-December 2018 including 26 epileptic patients (11 male/15 female) admitted to the Centro de Referência de Epilepsia Refratária of Centro Hospitalar e Universitário de Coimbra. Demographic data were collected as well as the AEDs co-administered and plasma concentrations at the first day and during the admission. Abbottbase PKS software was used to estimate levetiracetam volume of distribution (Vd) and clearance (CL). Patients were considered non-adherent if the absolute difference between predicted and observed plasma concentration of levetiracetam was ≥ 30%. All statistical analysis was performed using Statistical Package for the Social Science software.

The mean values of levetiracetam Vd and CL were 0.799L/kg and 0.067L/kg/h, respectively, exhibiting variation coefficients of 47% and 34%. Co-medication with enzyme-inducing antiepileptic drugs (EIAED) increased the Vd (0.922L/kg) and CL (0.084L/kg/h) of levetiracetam. Only twenty of the 26 screened patients were included in the adherence assessment and 50% of them were non-adherent.

High inter-individual variability was observed on the pharmacokinetic parameters of LEV. Polytherapy with EIAED revealed to have a significant impact in the pharmacokinetics of levetiracetam and, hence, this drug interaction should be monitored. This study also highlights the poor patient compliance to levetiracetam.

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P122-F | Design of a risk-stratification model for prediction of life-threatening ventricular arrhythmia's in patients with non-ischemic dilated cardiomyopathy

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Introduction: Patients with Non-ischemic Dilated Cardiomyopathy (NIDCM) are at risk of life-threatening

ventricular arrhythmia's (LTVA) and may benefit from an implantable cardioverter defibrillator (ICD). However, identification of these patients remains challenging. Several predictors have been associated with the risk of LTVA. Nonetheless, there is still no definitive risk-model for predicting LTVA in this population. Introduction of a risk-stratification model could help identify patients at risk for LTVA.

Objection: Here, we present the design of a risk-stratification model for primary prevention of sudden cardiac death (SCD) due to LTVA in the NIDCM-population.

Material and methods: NIDCM Patients will be selected from UNRAVEL Research Data Platform and through manual search of Electronic Health Record (EHR) by ICD10 codes. Clinical quantitative data are extracted automatically from the EHR. RedCap will be used for designing an electronic case-record form (eCRF) and is available on www.unravelrdp.nl. Based on previous literature, candidate predictors have been selected for analysis (e.g. ECG, MRI, Ultrasound, Laboratory). In order to maintain adequate precision a minimum of 10 events will be required per regression coefficient. Univariate and multivariate cox proportional hazard models with backward selection will be used to select predictors. We estimate to include ± 600 patients and subsequently select a maximum of 7 independent predictors to include in the risk calculator.

As primary outcome we will assess the time to first LTVA after diagnosis, defined as a composite of ventricular fibrillation (VF), sustained ventricular tachycardia (VT) >100 beats per minute for >30 seconds, sustained ventricular tachycardia with haemodynamic instability or electrically/chemically cardioverted VT/VF, (aborted) SCD or appropriate ICD intervention for LTVA.

Conclusion: This study aims to develop a risk-stratification tool for developing LTVA in NIDCM. This risk model could be useful for guiding physicians in patient selection for ICD implantation. The model will be validated in an external cohort.