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COVID-19 and Social Inequalities in a Syndemic Approach: Social, Clinical and Psychological Aspects

56ASM – 0278 PC05 | Changes in mindfulness during the first lockdown from COVID-19

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The Lockdown due to the pandemic from COVID-19 has been a cause of psychological and cognitive distress, generating insecurity, confusion and emotional isolation. Emerging adults were the most vulnerable to the psychosocial effects of the pandemic, also experiencing increased levels of rumination, intrusive thoughts, and cognitive failures. The awareness that emerges from paying attention to the present moment experience can prevent anxiety and fear associated with the lockdown, and decrease negative, ruminative, and automatic thoughts.

The aims of the present longitudinal study were (a) to establish pre- and during-COVID-19 mindfulness profiles in emerging adults; (b) to investigate how COVID-related emotions, socio-economic status and housing conditions influenced profile membership; and (c) to investigate whether there were differences across mindfulness profiles with respect to cognitive failures, intrusive thoughts and rumination.

Using a sample of 181 healthy emerging adults, the results showed overall more cognitive failures and rumination during the first lockdown, especially for those participants who have experienced a reduction of mindfulness levels. On the contrary, all those who showed an improvement in their level of mindfulness, reported stable or even diminished signs of cognitive distress. Financial difficulties and a reduced sense of privacy were predictive of less stability in mindfulness profiles. Thus, being fully aware about what is happening in the present moment can reduce cognitive discomfort and psychological maladjustment, in times of severe stress, such as those of social isolation that produce a strong sense of fragility and uncertainty.

56ASM – 0279 PC05 | Individual differences modulate social distance during COVID-19 pandemic

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Background: Interpersonal space (IPS) is the space we maintain between ourselves and others to feel and interact in a comfortable way. Individuals regulate IPS through basic behaviors, i.e., extending/reducing distance when they feel in dangerous/safe situations, respectively. Individual differences (e.g., sex, age, education level) and psychological factors (e.g., anxiety, fear, personality factors, risk perception) might modulate IPS. COVID-19 pandemic has obliged people to maintain larger-than-usual interpersonal social distances, along with wearing a face mask.

Aims: To investigate the effects of a) individual differences (sex, age, education level) and b) psychological factors (state and trait anxiety, local and general risk of contagion perception) on IPS during the first lockdown due to COVID-19 in Italy.

Methods: The survey was conducted online over April 2020 during the first lockdown. A total of 210 participants (164 females) aged 18 to 70 years took part in the study. The Interpersonal Visual Analogue Scale (IVAS) investigating the desired IPS distance maintained by the participant with respect to a variety of confederates (male and female; child, young, and old) and a battery of standardized self-report questionnaires investigating state and trait anxiety, and local and general risk of contagion perception were administered.

Result: The IPS distance regulation was affected by gender of the participants and age of the confederates. Interaction effects emerged between these variables, and between gender and age of the confederates. Moreover, IPS distance was predicted by state anxiety and perceived local risk of contagion.

Conclusions: These results seem to be in line with proxemics literature before and during pandemic and reveal

how subjective and psychological factors modify choices regarding the management of our own interpersonal space, even during a dangerous and life-threatening event such as a pandemic.

PLENARY SPEAKER: FRIDAY 10TH of JUNE

56ASM-0300 PS | Genetic factors predisposing to irritable bowel syndrome

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Irritable bowel syndrome (IBS) affects a large fraction of the general population with chronic symptoms including constipation, diarrhea, bloating and abdominal pain. Etiology is mostly unknown, and the pathophysiology likely involves several factors including genetic predisposition. Until recently, genetic research in IBS has been scarce and mostly characterized by small underpowered studies. This is now rapidly changing thanks to the availability of genomic and health-related data from large international biobanks, which offer unprecedented opportunities for powered genetic studies also in IBS. In addition to classical case-control studies, these resources allow investigations to be carried out using reliable endophenotypes that can be studied as proxies of gut function, like stool frequency and consistency. By means of nutrigenetic studies, genome-wide association studies (GWAS) and their meta-analyses, we recently provided compelling evidence for the importance of genes involved in the digestion of carbohydrates, ion channel function, neurotransmitters and their receptors, neuronal pathways, and the control of gut motility in IBS. We have also shown that IBS shares its genetic architecture with often comorbid mood disorders and anxiety, which may be relevant to therapeutic strategies based on the use of psychotropic drugs. Finally, polygenic scores computed based on recent stool frequency GWAS summary statistics allowed the identification of individuals exposed up to 5 times increased risk of IBS characterized by diarrhea. These findings can hopefully be translated for patients stratification and improved therapeutic efficacy in IBS.

56ASM–0326 PS | Inflammasomes as a gateway to autoinflammation

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Inflammasomes are central nodes of the innate immune system that promote production of the inflammatory cytokines interleukin (IL)-1β and IL-18. In addition, inflammasomes induce cleavage of Gasdermin D to induce an lytic cell death mode termed pyroptosis that is associated with release of dager-associated molecular patterns (DAMPs). Uncontrolled inflammasome activation has been identified as a key mechanism driving detrimental autoinflammatory diseases. Here, I will review current understanding of how altered inflammasome signaling contributes to inflammatory pathology based on studies with patient samples and preclinical models of autoinflammatory diseases. I will also highlight recent work from my laboratory illustrating how such mechanistic insights are being translated into novel therapeutics and diagnostics for autoinflammatory diseases.

SYMPOSIUM 1: THE GUT-LIVER BODY AXIS & LIFESTYLES: LESSONS FROM FRONTLINE BIOMEDICAL RESEARCH

56ASM-0285 S1 IS | Different variations of intrafamilial body mass index subjected to COVID-19 lockdown

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Background: The COVID-19 lockdown has represented an inedited model of increased metabolic risk in all age

groups, due to negative changes in dietary habits, physical activity, lifestyle. These effects have been generally explored at a population level in distinct age groups. Potential intra-familial, specific effects in adults and children sharing the same socio-economic, cultural level and living habits have been scarcely explored. We aimed to characterize changes of anthropometric indices in parents and in their children during lockdown.

Methods: A cohort of 149 couple parent/children were prospectively enrolled. By a validated questionnaire we explored changes of Body Mass Index (BMI) and individual lifestyle during a 2-month lockdown (May-July 2020).

Results: BMI increased in 70.5% of parents and in 67.8% of their children, with a D-BMI of 1.44 + 0.09 kg/m^2 and 0.36 + 0.02 Kg/m^2, respectively. BMI increments, however, were only significant in adults and did not correlate in the couple parents/children. Most adults (80.5%) and children (71.4%) did not perform regular physical activity during the lockdown. Direct correlations between dietary changes and BMI variations became evident in children, mainly in terms of decreased consumption of fresh fruit, pulses, fish, and increased consumption of cereals, carbohydrates, dairy products, olive oil. In normal weight, overweight and obese children, but not in adults, the increase in sleep hours increased with BMI.

Conclusions: Despite marked lifestyle changes imposed by the lockdown, BMI variations in parents were independent from those observed in their children, pointing to different outcomes in response to the same external, critical event. Thus, primary prevention measures aimed at maintaining a healthy lifestyle require different approaches according to age.

56ASM-0284 S1 IS | Effects of temporary sacral nerve stimulation on gastrointestinal motility and function in patients with chronic refractory slow-transit constipation

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Background: The efficacy of sacral nerve stimulation (SNS) on patients with chronic refractory slow-transit constipation is controversial and its mechanism of action on gastrointestinal motility and transit is not fully understood. The aim of this study was to document the effects of temporary SNS on gastrointestinal and biliary tract motility and on gastrointestinal transit in patients with refractory slow-transit constipation.

Methods: This was a prospective interventional study. Patients with slow-transit chronic constipation, unresponsive to any conservative treatment, were enrolled between January 2013 and December 2018. Patients' quality of life [patient assessment of constipation quality of life (PAC-QOL) questionnaire], constipation scores (Cleveland Clinic Constipation Score) colonic transit time (CTT), orocecal transit time (OCTT), gastric and gallbladder kinetics, together with the assessment of the autonomic nerve function were evaluated before and during temporary SNS.

Results: 14 patients (12 females, median age 38 years, range 24–42 years) had temporary SNS. The Cleveland Clinic Constipation Score did not change compared to baseline (23 ± 3 vs 21.4; $p = 070$). The PAC-QOL did not improve significantly during the stimulation period. Gallbladder/stomach motility (half-emptying time) did not change significantly before and after SNS. OCTT was delayed at baseline, as compared to standard internal normal values, and did not change during SNS. CTT did not improve significantly, although in two patients it decreased substantially from 97 to 53 h, and from 100 to 65 h.

Conclusions: Temporary SNS did not have any effect on upper/lower gastrointestinal motility and transit in patients with severe constipation

56ASM-0282 S1 IS | Reduced hepatic phospholipid (PL) output dramatically promotes the formation of cholesterol (Ch) gallstones in mice

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Background: The ATP-binding cassette transporter B4 (ABCB4) expressed on the canalicular membrane of hepatocytes plays a critical role in hepatic PL secretion.

Hypothesis: Disruption of the *Abcb*4 gene increases susceptibility to gallstones because of deficiency of biliary phospholipids in Abcb4 KO mice fed even a low Ch diet compared to wild-type (WT) mice.

Methods: The effect of deficiency of biliary phospholipids on Ch crystallization and gallstone formation was studied by physical-chemical methods in male Abcb4 KO vs WT mice fed a chow diet (on day 0) and a special diet

containing 0.1% Ch, 15% butterfat and 0.25% cholic acid for 4 wk.

Results: On chow (day 0), biliary phospholipids are 18.1 ± 0.3 mM in pooled gallbladder bile of WT mice. However, no phospholipids are detected in Abcb4 KO mice fed either the chow or the special diet. After 1 wk of feeding the special diet, phase diagram analysis finds that lipid composition of pooled gallbladder bile of Abcb4 KO mice is located in crystallization region A. Moreover, many needle-like Ch crystals, typical Ch monohydrate crystals, sandy stones and small gallstones (0.1–0.2 mm in diameter), but not liquid crystals, are detected in bile by phase contrast and polarizing light microscopy. This shows that lack of phospholipids in bile produces anhydrous crystalline metastable intermediates that rapidly evolve to Ch monohydrate crystals from supersaturated bile. In contrast, lipid composition of pooled gallbladder bile of WT mice is still within the micellar zone, indicating that bile is unsaturated with Ch. After 4 wk on the special diet, lipid composition of pooled gallbladder bile of Abcb4 KO mice still stays in crystallization region A, with many larger gallstones (0.3–0.6 mm) being formed in the gallbladder. Notably, lipid composition of pooled gallbladder bile of WT mice enters crystallization region C, as analyzed by phase diagram. Thus, many liquid crystals (i.e., vesicles) and classical solid plate-like Ch monohydrate crystals, but not gallstones, are found in the gallbladder of WT mice, indicating that solid Ch crystals are formed through the liquid crystalline pathway.

Conclusions: Lack of biliary phospholipids induced by disruption of *Abcb*4 in the liver enhances Ch cholelithogenesis by dramatically reducing Ch solubility in bile through the liquid crystalline pathway and promotes rapid Ch crystallization via the anhydrous crystalline pathway. Our findings provide novel insights into the pathophysiological mechanisms elucidating why Ch gallstones are rapidly formed in patients with the *ABCB*4 mutations, leading to low phospholipid-associated cholelithiasis, a rare biliary disease caused by a single-gene mutation.

56ASM-0283 S1 IS | Novel insight into the role of gut microbiota in the pathogenesis of cholesterol gallstone disease

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Background: While basic and clinical studies over the past decades have recognized that intestinal factors play an important role in the pathogenesis of cholesterol gallstone formation, mounting mouse and emerging clinical evidence indicates that the ecosystem of the intestine consists of trillions of bacteria forming a bioreactor that is fueled by dietary macronutrients to produce bioactive compounds. These microbiota-derived metabolites can signal to distant organs in the body, which enables the gut bacteria to connect to the hepatobiliary system. Bile acids, a class of amphipathic steroids produced in the liver and extensively modified by the gut microbiome, are increasingly recognized as actors in promoting the formation of cholesterol gallstones. However, the molecular mechanisms underlying the role of intestinal flora imbalance in the pathogenesis of cholesterol gallstones remain elusive.

Materials and Methods: We summarize the advances in the role of gut microbiota in the pathogenesis of cholesterol gallstone disease in humans and mice.

Results: Primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), can be synthesized via two well-known pathways, i.e., the classical and the alternative pathways, respectively. Conversion of primary bile acids into secondary bile acids by gut microbiota involves three major groups of bacterial enzymes: bile salt hydrolases, hydroxysteroid dehydrogenases and dehydroxylation of unconjugated bile acids in the colon. Although many bacterial species carry out different dehydroxylation reactions, only 7α and β dehydroxylation results in the formation of the major secondary bile acids, i.e., deoxycholic acid (DCA) and lithocholic acid (LCA). Upon the lithogenic diet and before the onset of cholesterol crystallization, gallstone-susceptible C57L mice have displayed a dramatic increase in biliary DCA concentrations, which is significantly higher than that in gallstone-resistant AKR mice. Subsequently, increased DCA is coupled with hypersecretion of biliary cholesterol and a high cholesterol saturation index (CSI), as well as rapid formation of biliary sludge and cholesterol gallstones in C57L, but not AKR, mice. Human studies also show that there is an

increase in the ratio of dihydroxy bile acids (DCA and CDCA) to trihydroxy bile acids (CA) in subjects with gallstones, which is coupled with high CSI. In vitro model bile studies found that DCA-enriched bile shifts all crystallization pathways to the right that favors rapid cholesterol nucleation and crystallization, thereby promoting the formation of cholesterol monohydrate crystals and gallstones. Consumption of the synthetic, unabsorbed disaccharide lactulose leads to acidification of the colon, thus inhibiting bacterial 7α-dehydroxylases which are inactive at pH < 6 and subsequently lowering the percentage of DCA in bile acid pools and reducing CSI. The ingestion of live *Streptococcus faecium* has similar effects on bile, probably by displacing the anaerobic organisms in the colon and inhibiting conversation of DCA.

Conclusions: Bile acids are synthesized in the liver and modulated by gut bacteria in the intestine. The gut microbiome and bile acid signaling pathways have emerged as attractive therapeutic targets for the prevention and treatment of cholesterol gallstones. These mouse and human studies strongly suggest that to modulate the microbiome-bile acid axis, fecal microbiota transplantation or probiotics/synbiotics should be further explored.

Keywords: biliary sludge; hepatic cholesterol hypersecretion; cholesterol crystallization; deoxycholic acid; microbiome-bile acid axis.

56ASM-0302 S1 IS | Therapeutic targets in inflammatory bowel disease

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Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a chronic-relapsing inflammatory disorder which primarily affect the gastrointestinal tract causing disabling symptoms lifelong. While no definite cure exists for this condition, over the last two decades the treatment paradigm has radically changed thanks to the introduction of monoclonal antibodies and small molecules into the market. For many years, the mainstay therapy has been represented by steroids, which have a pleiotropic and non-specific effect by suppressing several inflammatory pathways, and azathioprine, which inhibits purine synthesis, thus unselectively suppressing white blood cells. The first monoclonal antibody approved for the treatment of IBD was infliximab, an anti-tumour necrosis factor (TNF) α, which is a key cytokine implied into the triggering and amplification of mucosal inflammation, by recruiting both B and T cells and by inducing epithelial cell apoptosis. This cytokine is also implied in the pathogenesis of other immune-mediated conditions, including, among others, rheumatoid arthritis, psoriasis, arthritic psoriasis, and uveitis. Other anti-TNFα agents have been progressively licensed, namely adalimumab, golimumab, and certolizumab. The first gut-selective biological agent to be developed was instead vedolizumab, an anti-integrin targeting α4β7, inhibiting T-cell gut homing. Finally, the anti-IL12/23 monoclonal antibody ustekinumab, which had already been licensed for the treatment of psoriasis and its related arthritis, also proved effective in treating IBD. By blocking IL12/23, Th1 and Th17 responses are inhibited, thus promoting mucosal healing within the inflamed gut mucosa. Finally, Janus kinases (JAK) have been exploited as a target of small molecules, such as tofacitinib, which inhibits JAK 1 and 3, altering DNA transcription, while sphingosine-1-phosphate receptors agonists, such as ozanimod, act by inhibiting cell trafficking. Other druggable targets will soon be assessed, including other interleukins (e.g., IL10, IL13, IL12, IL23), T-cell surface proteins (e.g., CD25, CD4, CD40L, NKG2D), and other JAK inhibitors.

56ASM-0311 S1 IS | Accelerating healthy lifestyles: the case for Tobacco Harm Reduction

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Tobacco harm reduction (THR) is a public health strategy to prevent or reducing morbidity and mortality from tobacco use among smokers. While eliminating exposure to toxic chemicals and carcinogens generated by tobacco combustion would result in the greatest reduction of harm, THR acknowledges that this is not always achievable, and users may not always be able or willing to quit. So THR advocates that smokers switch to less harmful forms of nicotine consumption.

With the growing popularity of combustion-free nicotine delivery technologies, such as e-cigarettes (ECs) and heated tobacco products (HTPs), product substitution is now an important aspect of THR, with the aim of reducing health damage associated with combustible tobacco cigarettes.

Compared with conventional cigarettes, ECs and HTPs offer substantial reduction in exposure to toxic chemical emissions and, for this reason, they are proposed for harm reduction from cigarette smoking, for smoking cessation and for relapse prevention.

Cigarette substitution with combustion-free nicotine delivery technologies (i.e. ECs or HTPs) may reduce health damage associated with tobacco smoke. Therefore, it is expected that switching away from combustible tobacco cigarettes would produce significant health improvements and proof-of-concept studies are now emerging. This is consistent with what we have learned over the last 50 years about tobacco smoke chemical composition and respiratory disease pathogenesis.

More high quality work is necessary to quantify the relative risk of using these emerging technologies compared to cigarette smoking, to accurately establish product quality and safety in absolute terms, and to document health improvements in vulnerable populations (e.g. asthma, COPD, schizophrenia).

56ASM-0314 S1 IS | Lymphocyte subsets as a predictor of severity and mortality in COVID-19 patients

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Background: Coronavirus disease 2019 (COVID-19) still represents a worldwide health threat. Early prediction of the severity of the disease is important for reducing the death rate and controlling the infection. Previous studies indicated changes of peripheral lymphocytes in COVID-19 patients, but the prognostic value remains unclear. We aimed to describe the characteristic of blood cell counts and lymphocyte subsets in patients with COVID-19 in order to provide new clues for the use of this parameter for mortality prediction.

Methods: A total of 410 patients confirmed with COVID-19 in Policlinico Hospital in Bari Italy from September 2020 to May 2021 were included. Clinical data were collected and analysed. Diagnostic and prognostic utility of blood cell counts and lymphocyte subsets in COVID-19 patients were investigated. The receiver operator characteristic curve (ROC) was used in discriminating the probability of exitus in COVID 19 patients.

Results: There were difference in blood cell counts and lymphocyte subsets among mild, moderate, severe and critical patients, which were also influenced by comorbidities, duration of disease, age but not by gender of patients (even if severity in females was higher at more advanced age in comparison to males). CD45, T CD3, T CD4, B CD19 were significantly lower and NK CD16/56 CD3- (%) significantly higher at the onset of diseases in exitus patients (43/367). The area under the ROC of lymphocyte, CD3+ T cells, CD4+ T cells, and CD8+ T cells confirmed the prediction of mortality with sensitivity higher than 0,70. Patients with higher counts of lymphocyte, CD3+ T cells, CD4+ T cells, or CD8+ T cells were correlated with shorter length of stay in hospital. **Conclusion**: Blood cell counts, and lymphocyte subsets correlated with severity of COVID-19. Dynamic monitoring blood cell counts, and lymphocyte subsets might be helpful to evaluate severity and guide treatment of the patients.

56ASM-0315 S1 IS | Brown Adipose Tissue: Does it protect against the development of diabesity?

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Obesity represents the most prevalent metabolic disease worldwide and has become one of the main causes of death, since it constitutes the main risk factor for a series of comorbidities, in particular type 2 diabetes (T2D). This close relationship led to the coining of the term "diabesity" a few years ago, highlighting the fact that most people with T2D also have obesity. Brown adipose tissue (BAT) is a specialized type of adipose tissue that plays an important role in the regulation of body temperature. It is present in significant amounts in rodents and hibernating animals. In humans, BAT is well developed in the neck and interscapular region in the new-born. Until a few years ago, it was generally accepted that BAT involutes during the first months of life, with recognizable depots having disappeared within the first years after birth. However, recent findings have shown evident areas of BAT in adult humans mainly located in the supraclavicular region and neck, with additional activity in the paravertebral, mediastinal, para-aortic, and suprarenal areas. The activity of this BAT can be acutely triggered by cold exposure and is controlled by the sympathetic nervous system. Moreover, beige or brite adipocytes, brown adipocytes in white adipose tissue, have been shown to be involved in energy homeostasis. Evidence from mice suggest that transplantation or activation of BAT or/and

beige adipocytes may have an effect on energy homeostasis reversing insulin resistance in peripheral tissues and improving glucose homeostasis via several mechanisms. Furthermore, several BAT-derived endocrine regulatory molecules (brown adipokines or batokines) mediators of the beneficial metabolic effects of BAT activation have been identified. Consequently, stimulation of the activity of BAT has been proposed as a novel potential strategy for treatment diabesity in humans.

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Keywords: Brown adipose tissue, Browning, Diabesity, Obesity, Type 2 diabetes

56ASM-0334 S1 IS | Sedentary lifestyle habits predispose to higher risk of NAFLD: A Foie Gras study

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Background: Alongside with the increasing incidence of obesity and type 2 diabetes, also the non-alcoholic fatty liver disease (NAFLD) is growing. NAFLD by affecting 25% of adult population worldwide is becoming the significant burden of liver-related outcomes. Physical activity plays a crucial role in metabolic control and alongside weight loss and diet change it is recommended as therapeutic approach for people with non-alcoholic fatty liver disease (NAFLD). We aim to study the link between the sedentary lifestyle habits and NAFLD in our cohort from Southern Italy.

Materials and Methods: In Ninety-five subjects (age: 44.3 ± 1.4 years, body mass index: 28.6 ± 0.7 kg/m2, female: 46 %, NAFLD: 63.2 %,), liver steatosis (degree: 0–3) were graded and assessed by ultrasound (Noblus® Hitachi, 3.5 and 7.5 MHz probes, Italy). Physical activity was assessed using a self-reported International Physical activity questionnaire, long format version and levels were calculated based on Metabolic Equivalent Tasks (METs; 1 MET = 3.5 mL/Kg/min of oxygen consumption or 1.5 Kcal/Kg/hr).

Results: Mean degree of liver steatosis in NAFLD subjects was 1.6 ± 0.1 (mean ± SEM). When grouping subjects by the presence of NAFLD (healthy and NAFLD), NAFLD subjects reported significantly higher sitting time (910.4 ± 101.1 vs. 485.4 ± 47.5 min/day; $P = 0.00004$), performed less light physical activity (800.2 ± 152.3 vs. 1699.5 ± 472.7 METs/week, $P = 0.03135$), similar levels of moderate, vigorous, and total physical activity (652.3 ± 209.3 vs. 938.7 ± 231.7, 640.7 ± 213.9 vs. 601.4 ± 163.2, and 2080.6 ± 404.9 vs. 3239.7 ± 562.8 METs/week, respectively) than healthy subjects. Furthermore, NAFLD reported less energy expenditure than healthy subjects (2985.5 ± 574.3 vs. 3680.6 ± 674.4 kcal/week) however, this finding was not significant.

Conclusions: In our clinical setting, subjects with NAFLD spent more time sedentary and undertake less physical activity levels on weekly basis than healthy. Almost above 40% of our study population did not meet the required minimum physical activity levels emphasizing the need for more encouraging advice from physicians and healthcare personnel.

56ASM-0335 S1 IS | Systematically Reviewing the Effects of Probiotics in Psychosocial Stress

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Background: The burden of disease depending on mood and psychological symptoms is important in the daily practice of clinical medicine. Probiotics are useful in several conditions characterized by gut dysbiosis, bacterial overgrowth, intestinal inflammation, irritable bowel symptoms etc. Probiotic supplementation have been reported to provide beneficial effects on mood and psychological symptoms. Target conditions can be stress and anxiety disorders. Most studies provide data based on animal models and clinical data do not reach consistent conclusions.

Aim: We designed a systematic review to explore if and to which extent probiotics can influence psychological stress and anxiety disorders.

Methods: Databases including Medline, PubMed, Scopus, Web of Science and clinicaltrials.gov were searched on the Web up to 2022. Included were only double-blind, randomized and placebo-controlled or prospective studies. We also reviewed studies measuring preclinical psychological symptoms of perceived stress and anxiety, before and after supplementation with a probiotic.

Results: We found 12 studies meeting the inclusion criteria, dealing with stress ($N = 3$), anxiety (N = 4), or both (N = 5). Overall, the studies had included 1,521 participants. Improvement of stress was reported in 33% of the

studies, improvement of anxiety in 75% of the studies, and improvement of both stress and anxiety was reported in 40% of the studies.

Conclusions: Not many studies have systematically addressed the effects of probiotics on psychosocial stress, namely stress, anxiety, or both. Available evidence suggests that probiotics may improve a few psychological symptoms associated particularly with anxiety. Further research needs to investigate to which extent probiotics can improve additional psychological stress disturbances.

56ASM-0336 S1 IS | Beneficial Effects of a Novel Nutraceutic in the Improvement of Gastrointestinal Symptoms and Habits in Subject with Irritable Bowel Syndrome: A Pilot Study

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Background: Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders which affects 10-25% of the general adult population. IBS is characterized by recurrent chronic abdominal pain and altered bowel habits as constipation (IBS-C) and/or diarrhea (IBS-D) in the absence of organic damage. We aimed to evaluate the effect of a new nutraceutical on symptoms and gastrointestinal habits of IBS subject.

Methods: We enrolled 16 patients (9 men, 7 women). The initial workup started by the exclusion of organic diseases. The diagnosis of IBS was made according to ROME IV criteria. Thus, we enrolled 12 subjects with IBS-C, and 4 subjects with IBS-D). Patients underwent the administration of a novel nutraceutical for 4 months, 2 pills/day for one week and after 1 pill/day for 11 weeks containing in an open fashion study. The nutraceutical contained *Lactobacillus acidophilus* LA14, billion/CFU and *Bifidobacterium Longum* BL04, 1 billion CFU, L-Tryptophan, Inulin, Charcoal, Mint, Lemon balm, Chamomile, Licorice and Vit B (*Colonir*, Omega Pharma, Cantù, Italy). At the beginning and at the end of the study the following parameters were evaluated: intensity of the abdominal pain (VAS 0–100 mm), number of days with abdominal pain, bloating, bowel habits by Bristol stool chart.

Results: According to the body mass index (BMI) subjects were moderately overweight (25.7 ± 3.9 Kg/m^2). After treatment, patients with IBS-C displayed a reduction of the number of days with abdominal pain (from 5.6 ± 2.4 to 3.3 ± 2.6 days, $P < 0.001$), improvement of bloating (from 73 ± 17 to 55 ± 20 mm, $P = 0.01$), and bowel habits (constipation) by Bristol stool (score from 1.9 ± 0.6 to 3.9 ± 0.4, $P < 0.0001$).

Conclusions: The novel nutraceutical is effective in improving symptoms and intestinal habits in IBS-C subjects. Further controlled, double-blind studies are required to confirm these results in a large population according to all the subtypes of IBS.

56ASM-0337 S1 IS | Gender Differences in Psychological and Metabolic Effects Post COVID-19 Strict Social Distancing Measures

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Several nationwide restrictions have been adopted to block the COVID-19 pandemic leading to indoors confination of millions of people. These measures led to changes in lifestyle behaviours. The lockdown impaired the global public health status since the prevention and follow-up of non-communicable diseases were limited to virtual consultations. The main consequences were the increased prevalence of cardiometabolic diseases, and the dangerous onset of psychological disorders. Several observational and epidemiological studies revealed pervasive concerns about the effect of social distancing and health measures during COVID-19 lockdown that infringe on personal freedoms on psychophysical health. Women especially suffered from depression and anxiety during COVID-19 lockdown. Moreover, women displayed a worsening of obesity, type 2 diabetes, and visceral adiposity associated with chronic, low-grade systemic inflammation or meta-inflammation. These detrimental effects were more evident in married women with children which were affected by the care burden at home.

56ASM-0338 S1 IS | Exclusion Diets in Functional Dyspepsia (FD)

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Background and aim: Patients with FD represent a major group of subjects requiring attention, consultation, and therapy by several clinicians. Studies have been carried out to establish useful dietary recommendations for FD patients. The ultimate gain in this approach remains to be elucidated in FD patients. We conducted a systematic review to analyze the effect of exclusion diets in functional dyspepsia.
Methods: This systematic review was written following PRISMA guidelines. The electronic databases PubMed, EMBASE, and Cochrane Library were searched using this search string: ("Functional Dyspepsia" [Mesh]) OR ("Dyspepsia" [All Fields]) AND (("Diet" [Mesh]) OR ("Nutrition" [All Fields]) OR ("fermentable oligosaccharides, disaccharides, monosaccharides, and polyols" [Mesh]) OR ("FODMAP" [All Fields]) OR ("Gluten" [Mesh]) OR ("Lactose" [All Fields])). Observational studies assessing the impact of various diets on the evolution of functional dyspepsia were eligible for inclusion. Exclusion criteria: Experimental studies. Studies published in languages other than English; Case reports, letters, reviews, short surveys, practice guidelines, press articles, conference abstracts/papers, abstracts. Quality Assessment by NHLBI and Newcastle-Ottawa Scale (NOS).
Results: Results show that for any given symptom, clinicians were prone to advise for a variety of foods to exclude, depending on personal background.

Symptoms	Type of food
Early satiety	Red meat, bananas, bread, cakes, pasta, sausages, fried foods, beans, onions, mayonnaise, milk, chocolate, eggs, sweets, oranges
Bloating	Soft drinks, onions, beans, bananas
Epigastric pain Epigastric burning	Cheese, Coffee, onion, pepper, milk, chocolate, pineapple

Conclusions: Diverse attempts have been carried out to establish useful dietary recommendations for FD patients without gaining consistent results. Exclusion diets have not proved to be uniformly useful, as emphasized by our review. Dietary recommendations should not be dogmatic and should be based on individual tolerance. As a general rule, it is reasonable to advise frequent small-size meals and avoid high-fat food.

56ASM-0339 S1 IS | Diabetes Mellitus and the Gastrointestinal Tract: a deeper embrace

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Diabetes mellitus type 1 and type 2 are some of the commonest endocrine disorders. All tracts of the gastrointestinal system can become potential targets in diabetic patients. Dysfunctions can occur and cause signs and symptoms at different levels. In the esophagus, the most frequent manifestations include heartburn, gastroesophageal reflux, and motility disorders. The stomach can manifest diabetes-related abnormalities which include gastroparesis, tachygastria, myoelectrical disturbances with nausea, vomiting, fullness. The intestine is interested

during diabetes with chronic constipation or diarrhea and, rarely steatorrhea. Intestinal dysbiosis and malabsorption can represent additional features. The gallbladder can be one target of diabetes mellitus due to motility defects, stasis, precipitation of cholesterol crystals and cholesterol cholelithiasis. This condition is a predisposing factor for the development of gallstone-related symptoms and complications in diabetic patients. In addition, the dysregulated gastrointestinal tract can influence many metabolic aspects in diabetes mellitus.

During the workup of diabetic patients, clinicians must be aware that diabetes mellitus and its implications for the gastrointestinal tract.

56ASM-0007 | Wine Polyphenol Resveratrol and Non-Alcohol Fatty Liver Disease – What do we know so far?

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Background: The most effective treatment of non-alcoholic fatty liver disease (NAFLD) represents weight loss, improving in insulin resistance and redirection of metabolic pathways of lipids towards catabolic processes. Previous findings have indicated that natural polyphenol resveratrol (RSV) mimics a condition of calorie restriction (CR) and, through activation of key regulators of metabolism - adenosine monophosphate-activated kinase (AMPK) and silent information regulator 1 (SIRT1), enhances glucose uptake in the skeletal muscle and stimulates β-oxidation of fatty acid. This can be crucial in the prevention of further progression of NAFLD. AMPK-dependent RSV action is also associated with the decrease in lipogenesis-associated genes, leading to a reduced lipogenesis and liver fat accumulation. The beneficial effects of RSV may also be due to its ability to modulate hepatocellular apoptosis. However, at a high dose (20 mg/kg) RSV can aggravate the liver injury, increasing the aminotransferase release, decreasing the antioxidative enzyme action and reducing the GSH levels, thus its final effects remain elusive. The aim of this review is to bring the data from clinical trials suggesting hepatoprotective effects of RSV and its potential use in the treatment of NAFLD.

Materials and Methods: We have conducted a literature search for the period of 1.1 - 31.12. 2020 year to identify a breadth of quality references regarding clinical data on hepatoprotective effects of RSV. We have searched "PubMed", "Scopus" and "Google Scholar" typing the following keywords: "liver", "hepatoprotective", "resveratrol", "clinical trials". Studies should be written in English language.

Results: Based on a clinical trials, the effects of RSV on liver enzymes (AST/ALT) and lipid profile appears to be inconsistent. Some studies suggest an increase in the LDL and total cholesterol but no differences were confirmed on the insulin resistance, systolic pressure, steatosis grade or abdominal fat distribution compared to a baseline.

Conclusions: Due to inconsistencies in the existing scientific reports on the RSV effects on NAFLD-related parameters, the need for further research is necessary. Additional carefully designed clinical studies need to be undertaken to provide scientific evidence for the efficacy of NAFLD.

56ASM-0033 | Mechanisms for maintaining postural stability in the static test with head turns

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Background: The static postural-tonic reflex (position reflex) when turning the head ensures the redistribution of muscle tone depending on the position of the body in space and ensures the balance of the body. In an adult, positional reflexes are difficult to assess visually due to the strong control from the suprastem structures of the central nervous system. The purpose of the work is to determine the change in plantar pressure during the implementation of a static cervico-tonic reflex to head turns in healthy subjects using the plantography method.

Materials and Methods: The study involved 31 healthy subjects aged 19 to 23 years. A plantographic study was carried out lasting 1 minute in a standard position (head straight), as well as when turning the head to the right and left. The pressure of the left and right foot was determined and the coefficient of lateral asymmetry was calculated.

Results: According to the coefficient of asymmetry in the standard position, the subjects were divided into 2 groups: in 19 subjects, the pressure of the right foot prevailed, in 12 subjects, the pressure of the left. In subjects with right-sided lateralization, when turning the head to the left, only a tendency to increase the pressure of the right limb was revealed, when turning to the right, a significant increase

in pressure of the contralateral (left) leg was observed on average by 10%, moreover, in 10 subjects, a change in lateralization was observed-pressure was transferred to the left foot. Subjects with a predominance of left foot pressure, turning their heads to the right, showed a tendency to increase the pressure of the left foot; turning the head to the left (in the direction of predominance of pressure in the background) led to an increase in the pressure of the right limb by an average of 7%, the contralateral transfer of body weight to the right limb was recorded in 4 out of 11 subjects.

Conclusions: Thus, plantography makes it possible to assess the implementation of a static postural-tonic reflex to head rotation in a person, while it is necessary to take into account the functional lateral asymmetry of the legs. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

56ASM-0046 | Lipid Lowering and Antioxidant Effects of an Ethanolic Extract from Sarcopoterium spinosum fruits on Steatotic hepatocytes

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Background: *Sarcopoterium spinosum*, a Mediterranean shrub of Rosaceae family, is used to treat diabetes and to relieve pain in folk medicine. Previous studies have reported the anti-diabetic activity of *S. spinosum* root extracts, however, the properties of aerial parts, especially fruits, have not been investigated. The aim of the present study is to investigate the potential of an ethanolic extract from *S. spinosum* fruits (SSE) as possible lipid-lowering and antioxidant agent.

Materials and Methods: Fruits of *S. spinosum* were subjected to ethanolic extraction. The SSE chemical composition was characterized by high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Total phenol content and *in vitro* radical scavenging ability were quantified spectrophotometrically. Finally, the *in vitro* beneficial effects were tested using rat hepatoma FaO cells overloaded with a fatty acids (oleate/palmitate 0.75 mM) and then exposed to increasing concentrations of SSE (1, 10, 25 μg/mL) for 24 hours.

Results: Triterpenes, tormentic acid especially, are the most abundant polyphenols in SSE that showed also a high TPC value (160.7 mgGAE/g dry extract) and an appreciable scavenging ability (IC_{50} of 15.9 μg/ml, and 10.9 μg/ml measured by DPPH and ABTS assays, respectively). In steatotic hepatocytes, SSE treatment significantly decreased the triglyceride accumulation as observed by both spectrophotometric assay and fluorescence microscopy. Furthermore, the fat-induced lipid peroxidation was significantly decreased upon SSE treatment, and SSE also restored the catalase antioxidant activity which was impaired in steatotic cells.

Conclusions: Taken together, our results indicate that the ethanolic extract from *S. spinosum* fruit exerts considerable lipid lowering and antioxidant activities on steatotic hepatocytes, maybe depending on the abundancy of triterpenes. Therefore, this extract from *S. spinosum* fruits could be a potential candidate for nutraceutical applications.

56ASM-0052 | Exploring metabolic disorders using a novel integrated approach to decipher the molecular alterations in hepatic and adipose cells

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The widespread epidemic of obesity is directly linked to increasing prevalence of non-alcoholic fatty liver diseases (NAFLD). Obesity is mainly consequence of overnutrition leading to hypertrophy of adipocytes that, in turn, release high levels of circulating fatty acids, triglycerides (TGs) and cytokines targeting hepatocytes and promoting NAFLD. Although numerous predisposing genetic and lifestyle factors have been identified, the biological mechanisms underlying the complex pathways linking adipose tissue hypertrophy and liver dysfunction are not fully elucidated. Furthermore, the interplay between genetics, epigenetics and the environment are largely involved in obesity. The development of cellular models to mimic *in vitro* what is occurring *in vivo* has become a trend in translational medicine. In this regard, our research group developed

well-established cellular models for hepatic steatosis and adipocyte hypertrophy using lipid-loaded hepatocytes and adipocytes. These *in vitro* models are investigated by a multiple novel integrated approach combining molecular and biochemical analyses, single cell force spectroscopy, and innovative optical approaches (Quantitative Phase Microscopy, Confocal and Super-Resolution Microscopy) to unveil the molecular and cellular mechanisms. In fact, the intracellular TGs are stored under form of cytosolic lipid droplets that are stiffer than the aqueous cytosol and might mechanically distort the cell and alter its elasticity and biomechanics. Through single cell force spectroscopy and label-free approach, we explored the potential role of mechanobiology in NAFLD. Recent evidence supports the role of epigenetics in adipocyte differentiation and hypertrophy. Using Super-Resolution optical approaches, such as STimulated Emission Depletion (STED) microscopy and specific immunoassays, we investigated chromatin architecture and epigenetic markers in the nucleus of adipocytes. The use of this interdisciplinary advanced approach to investigate reliable *in vitro* models of metabolic disorders paves the way toward a deeper comprehension of the multifaceted pathways linking the different tissues which interact in metabolic disorders.

56ASM-0057 | A Standardized in Vitro Digestion of Thymbra spicata L. Aerial Parts Modulates the antiproliferative Capacity of the Extracts

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Background: Thymbra spicata L. is an East Mediterranean species of the Lamiaceae family which has widely been used as food and in folk medicine. Leaves are rich in phenolic compounds and their extracts have shown to play several beneficial effects including antioxidant, lipid lowering, and anti-proliferative activities.
Materials and Methods: In order to assess how the digestion steps sequentially occurring in vivo in the mouth, stomach, and intestine might affect the phenolic compound profile and biological activity, two different extracts from T. spicata L. aerial parts were prepared using water (TW) or ethanol (TE) as solvents, before or after in vitro digestion (dig-TW and dig-TE, respectively) mimicking the in vivo process. All extracts were characterized in terms of phenol and flavonoid content and profile, protein and carbohydrate contents, in vitro antioxidant and in vivo antiproliferative activities, taking carvacrol as an internal control.
Results: Although each extract did not show significant differences before and after digestion, the total protein content was significantly higher in the low-molecular fraction of dig-TW. Moreover, a higher antioxidant potential was revealed in TW and TE after digestion. Finally, the dig-TE exhibited a more pronounced anti-proliferative activity on three human tumor cell lines and this effect paralleled the production of reactive oxygen species (ROS) and nitric oxide leading to cell-apoptosis.
Conclusions: These findings suggest that the physiological digestion may enhance some biological activities of T. spicata extract as it may supply the matrix with low-molecular fractions rich in non-extractable polyphenols naturally bounded to proteins and carbohydrates.

56ASM-0058 | Method for assessing motor activity by the capture system

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Background: The kinematic characteristics of the movement make it possible to describe the spatial displacements of the body and its individual links in space. Moreover, using kinematics data it is possible to solve the inverse dynamic problem to find forces that initiate these displacements. The purpose of this work is to develop and describe methods for assessing the movement of the object.
Materials and Methods: During motion capture, the rats began to move naturally in an open field. Spline interpolation was used to resample Vicon data up to 30Hz before analysis. Kinematic analysis was carried out for the complete gait cycle. To analyze the data obtained, the described method was implemented in the MATLAB software package. Three-dimensional data was obtained using six Vicon MX cameras. To analyze angle changes results were averaged for all steps. In this case, the resultingdistribution can be presented as follows: $\bar{\varphi} \pm (\tau) = \text{mean}(\varphi(\tau),\text{Nstep}) \pm \text{std}(\varphi(\tau),\text{Nstep})$ where $\varphi(\tau)$ -angle function, Nstep -number of steps.
Results: Calculated parameters such as step length, maximum foot lift, and foot swing give a clear indication about the subject moving. Also, the construction of angulograms is informative, the average values of the change in the angles of the hip and knee in steps and the standard deviation are calculated. Angulograms can be used to

determine gait in normal and pathological conditions, as well as before and after treatment.

Conclusions: The developed technique helps to accelerate the diagnosis of the subject's disease and personalize the treatment. The calculated parameters are indicative and give a clear picture of the nature of the subject's movement. This technique will be useful for tracking the dynamics of the subject's state. The proposed methodology is based on simple optimal algorithms and is easy to be automized. This work was part of KFU Program PRIORITY-2030 and funded by subsidy for the state assignment № 0671-2020-0059.

56ASM-0075 | Prevalence of Diseases of the Digestive System in the Child Population of the Republic of Tatarstan

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Background: Aim; to reveal the significance and the dependency factor of the digestive organs' diseases (DOD) prevalence in the child population aged up to 14 years on the parameters and the amount of pesticide use at the regional level

Materials and Methods: The analysis included data from the Center for Information and Analysis of the Ministry of Health of the Republic of Tatarstan (RT), the Ministry of Ecology and Natural Resources for the period of 2004 - 2018.

Results: In the Republic, DOD ranks 2nd among all diseases in children aged 0–14 years by year. For the years of 2004 – 2018, the primary DOD incidence went up 3.2 times. The major diseases were the gallbladder and biliary diseases (23.4–25.5 %), gastritis and duodenitis (14.1–18.5 %), functional gastric disorders (6.9–9.6 %). A two-fold growth of pesticide areas and volumes on simultaneous increase of glyphosate preparations: from 1.1 % (the year of 2002) to 26.5 9% (the year of 2016) and others, more than 66.3 % of which were multicomponent mixtures, resulted in the increase of all primary disease incidence of the child population, and to a greater extent, of DOD: for the period of 2004 - 2018 from 24.3 to 77.21 cases per 1000 children. Pearson correlation coefficient of new DOD incidence rate depending on the pesticide use area was equal to 0.88 ($p < 0.001$) at 95 % confidence interval (CI): 0.64÷0.96.

Conclusions: High prevalence of new DOD cases in children in the RT of the is caused by quantitative and qualitative differences in the proportion of the plough field processed with pesticides (29–94 %); the proportion of the major preparations (41.6–94.3 %).The relationship was observed for three years after exposure, and we understand this fact as the impact prolongation. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0121 | Gender Peculiarities of Adaptation of the Cardiovascular System of adolescents to Stress

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Background: Important mechanisms that adapt the cardiovascular system to changes in the position of the body in space is a change in the heart rate, which in adolescents is subject to large individual fluctuations and significantly depends on exogenous and endogenous factors. The aim of the study was to identify changes in the indicators of the cardiovascular system in adolescents with an active change in body position.

Materials and Methods: The methods Used an orthostatic sample. The object of the study is 120 adolescents 12–13 years old. Kazan.

Results: The active change of body position affected the changes in heart rate, the highest value was recorded in the experimental group (EG) (43.67 beats/min), which is more than the control group (KG) ($P < 0.05$).Close correlations between heart rate and anthropometric indicators were revealed in boys aged 12–13 years, the correlation coefficient in the sitting position in the EG was $P = 0.63$, KG - $P = 0.23$.

The highest indicators of UOC were determined in KG and an active change in Body position causes a significant decrease in this value - 19.90 ml ($p < 0.05$), in EG they amounted to 9.5 ml. High correlations of UOC and IOC in the supine position were established in the studied groups ($P = 0.92$ and $P = 0.94$), respectively, in both groups, close correlations of UOC with minute heart function (MPC) were recorded ($P = 0.71$).The average correlation coefficient ($P = 0.52$) and with the cardiac index ($P = 0.57$) was determined between heart rate and MRC. The indicators of specific peripheral vascular resistance have negative associations with UOC ($r = -0.58$).

Conclusions: In 44% of adolescents aged 12–13, the parameters of the functional state of the cardiovascular system do not correspond to age norms, indicating their insufficient adaptation to environmental factors, and represent a "risk" group. "This article was supported by the Strategic Academic Leadership Program of Kazan Federal University (PRIORITY-2030)."

56ASM-0142 | Gender Differences in Psychophysiological Parameters of Anxiety and Emotional Excitability

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Background: At the beginning of our study, we assumed that there is a difference in the mental, biochemical and physiological functioning of young people living in different areas of Kazan with different ecologies due to air pollution.

Materials and Methods: Indicators of reactive and personal anxiety in girls and boys using Spielberger.

Results: Analysis of the data allows us to identify stable relationships between anxiety and a number of physiological parameters in a stressful situation. In particular, there is a noticeable inverse correlation (according to the Chaddock scale) between anxiety and the functional state of the respiratory system (maximum flow rate achieved during forced exhalation, POS (peak volumetric velocity, l/s); lung capacity, VC (exp) ; instantaneous volumetric velocity at the time of expiration MOS25; forced expiratory volume FVC (normal)). A significant correlation was also found (according to the Chaddock scale) between anxiety and blood parameters (hemoglobin, hematocrit, erythrocyte index - MCHC, lymphocytes) and hormones (testosterone). In most cases, the correlation is inverse. Thus, an increase in anxiety leads to a decrease in all indicators. there are statistically significant differences (at $p < 0.001$) in the indicators of personal anxiety in the studied groups. In women, the average level of personal anxiety in the sample is higher than in men.

The mean values for the level of anxiety are statistically significantly different in the group of men and women, according to these data, women are more anxious than men. Testosterone levels show a significant correlation with personality anxiety.

Conclusions: The relationship between indicators of atmospheric air pollution and situational (reactive) anxiety, as well as emotional excitability indicates the influence of the environmental factor in the general stressful state of boys and girls. "This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)".

56ASM-0168 | The role of free fatty acid receptors in the effects of short chain fatty acids on mouse colonic contractility

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Background: Short chain fatty acids (SCFAs) are key products of fermentation of indigestible carbohydrates by commensal bacteria that reside in the gastrointestinal tract. Previously it was shown that SCFAs can participate in regulation of the colon peristalsis and different effects on gastrointestinal motility. Recently, the role of free fatty acid receptor 2 (FFA2) and FFA3, was suggested as the mechanism of SCFAs-related physiological responses. The aim of our study was to analyze the role of FFA receptors in the effects of butyrate on spontaneous contractions of the mouse colon.

Materials and Methods: Spontaneous contractions of the proximal colon segments of mouse were recorded under isometric conditions using an isolated organ bath system (Biopac, USA). During the experiment, the organ bath was filled with Krebs solution. Sodium butyrate was used in concentration 10 mM.

Results In control the proximal mouse colon demonstrated spontaneous activity after mounting the specimen.

Application of sodium butyrate induced decrease of tonic tension, amplitude and frequency of spontaneous contractions. Activation of FFA3 receptors - AR420626 (10 μM) didn't change the parameters of spontaneous activity. In this conditions the inhibitory effects of sodium butyrate on tonic tension, amplitude and frequency of spontaneous contractions were preserved and did not differ from its effects in the control. The inhibitor of FFA2 receptor - GLPG0974 (100 μM) also didn't change the parameters of colon contractility. Under these conditions, sodium butyrate on tonic tension and amplitude was preserved, however its effects on the contraction frequency were less pronounced compared to control.

Conclusions Our results demonstrated that FFA receptors are not involved in the effects of sodium butyrate on spontaneous contractile activity, but FFA2 receptors may partially mediate the inhibitory effect of sodium butyrate on the frequency of spontaneous contraction.

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56ASM-0173 | Aquaporins as emergent drug targets for obesity and cancer

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Since the discovery of aquaporins that research related to these membrane channels has never ceased growing. The classical water channels were found to have a much broader selectivity than initially expected, facilitating the transmembrane diffusion of many other small molecules, ions, and gases, and to be modulated by unique mechanisms allowing fine-tuning of their activity. In addition, aquaporin participation in cellular processes such as cell proliferation and migration highlighted their importance and their role as essential channels for life. A vast array of human disorders has been correlated with aquaporin dysregulation, missorting or mutations, unveiling their essential role in health and disease. The crucial role of aquaporins in kidney disease, brain oedema, metabolic disorders, obesity and cancer are examples of diverse pathological conditions where these proteins play a role. Here we present a few examples where targeting aquaporins may represent innovative therapies, unveiling their potential as drug targets for treatments and biomarkers for prognostic. Among the broad range of diseases where aquaporins are implicated, we are mostly interested in investigating their druggability for metabolic disorders (obesity, diabetes)[1], cancer[2], and inflammation[3, 4], aiming to provide a basis for the development of aquaporin-based therapeutics for these diseases.

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56ASM-0184 | Neurometabolic changes in the hippocampus of type 2 diabetic rats: an imaging perspective towards clinical applications

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Background: Type 2 diabetes (T2DM) induces neurologic comorbidities that include neurotoxicity induced by the imbalance between excitatory glutamatergic signalling and gamma-aminobutyric acid (GABA)-induced inhibitory activity. Our objectives were to evaluate the early alterations of such mechanisms in animal models and apply imaging methods that can ensure clinical application.

Materials and Methods: Experimental groups: 1) Control group of male Wistar rats only fed standard diet (SD, 10 weeks); 2) high fat diet (HFD)-induced obese rats; 3) Obese rats induced to T2DM by a low dose (35 mg/kg,

i.p., 4th week) of streptozotocin (STZ). Rats were subjected to magnetic resonance spectroscopy (MRS) and their hippocampus and visual cortex (VC) were collected for biochemical experiments.

Results: Glutamine, but not glutamate, levels are elevated in the VC of the HFD+STZ group, as well as in the hippocampus of the HFD group ($p = 0.0769$) and HFD+STZ group. Moreover, HFD potentiates the N-acetylaspartylglutamic acid (NAAG) formation, through the conversion of alanine, in the hippocampus, being also correlated to glutathione (GSH). Such suggests that NAAG prevents Glu release, redirecting it into the formation of GSH. However, GABA levels and $GABA_A$ receptor were not altered. No changes were observed for catalase, glyoxalase-1, heme-oxygenase and nitrotyrosine, nor the marker for cellular viability N-acetylaspartate (NAA).

Conclusions: In the hippocampus of the HFD model, Glu synthesis is not affected. Through the inhibitory effect of NAAG, Glu release into the synaptic cleft is apparently prevented in order to redirection the excitatory neurotransmitter into the formation of GSH as a compensatory mechanism. However, this effect is lost in the HFD+STZ model, showing the noxious effects of diabetes in the brain even before a compromised cellular viability. The metabolite NAAG may be a valuable early marker of neurologic comorbidities.

56ASM-0185 | Exposure to obesogenic environments during perinatal development modulates offspring nutrient-sensing pathways in adipose tissue

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Background: Obesogenic environments such as maternal obesity and westernized diets can impair the central and peripheral nutrient-sensing mechanisms of offspring during embryonic development and lactation. The dysregulation of energy balance regulators can compromise the insulin pathway and energy storage and therefore contributing to the development of metabolic syndrome at adulthood. Evaluate the insulin sensitivity in offspring exposed to maternal obesity and maternal glycation during perinatal period. With the second aim we intend to address the impact of these two obesogenic environments on peripheral and central signaling that regulate energy balance in newborn rats.

Materials and Methods: Two animal models were studied: 1) Offspring (42 days) of Sprague-Dawley dams submitted to a hypercaloric diet during pregnancy and lactation. 2) Offspring (45 days) of Wistar dams treated with S-p-bromobenzylglutathione cyclopentyl diester (BBGC, selective inhibitor of Glyoxalase-1, 5mg/kg, 6 days after delivery). DMSO was used as vehicle. Besides lipid and glycaemic profiles, NPY, ghrelin, dopamine and insulin pathways in white adipose tissue (WAT) and liver of offspring were analysed.

Results; The male offspring submitted to maternal obesogenic diet presented higher WAT levels of lipogenic [NPY receptor-1 (NPY1R)], but also lipolytic and catabolic mechanisms [dopamine-1 receptor (D1R) and p-AMPK], whereas phosphorylated insulin receptor was decreased. However, exposure to maternal glycation has the opposite effects on offspring's energy balance pathways, decreasing NPY1R levels, which is in accordance with the observed lower body weight and food intake in the descendance of BBGC-treated females. Regarding the liver, both NPY1R and D1R levels were decreased in both models.

Conclusions: Obesogenic environments induce compensatory mechanisms in WAT, increasing simultaneously lipid storage and oxidation. On the other hand, such mechanisms may be disrupted by exposure to glycotoxins during lactation, contributing to a greater predisposition for metabolic diseases later in life.

56ASM-0204 | Relationship between liver stiffness and steatosis: in vitro and in vivo studies

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Background: The first consequence of overnutrition is the excessive fat accumulation in the adipose tissue leading to overweight and obesity, being major public health

problems. Obesity is a major risk factor for various metabolic dysfunctions, including non-alcoholic fatty liver disease (NAFLD). The clinical phenotypes of NAFLD encompass from simple steatosis to non-alcoholic steatohepatitis (NASH) with varying degrees of fibrosis or cirrhosis. The aim of this study is to characterize the grade of steatosis being associated with overnutrition and obesity, both at the level of single hepatocyte and whole liver and to correlate it with the hepatocyte/liver stiffness and dysfunction.

Materials and Methods: The *in vivo* study enrolled 60 subjects being divided into three groups (healthy, moderate and severe liver steatosis) based on the stage of liver steatosis/fibrosis assessed by biochemical analyses, liver ultrasonography (AU) and acoustic radiation force impulse shear wave elastography (ARFI-SWE). For single-cell analyses, the three patient groups were mimicked by using *in vitro* models of moderate and severe steatosis on which to assess the single-cell biomechanics by Single Cell Force Spectroscopy (SCFS) and Quantitative Phase Microscopy (QPM).

Results: Results show that *in vivo* liver stiffness depends mainly on the extent of fat accumulation and not on fibrosis. These results parallel the *in vitro* observations showing that hepatocyte stiffness, morphology and dysfunction change with increasing the size of cytosolic lipid droplets.

Conclusions: Our findings indicate that the extent of steatosis affects markedly the biomechanical properties of both liver and single hepatocytes thus proving insights into the role of modulation of liver/hepatocyte elasticity as a physical mechanism transducing the obesity-dependent excess of plasmatic lipids towards liver steatosis and dysfunction.

56ASM-0208 | Neurometabolic and behavioural alterations in the adolescent offspring upon maternal glycation

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Background: Lactation period is an important programming window for the offspring predisposition to metabolic syndrome at adulthood but also to the modulation of neuronal alterations and behaviour later in life. The mechanisms of this intricate relation between metabolic and behavioural alterations but may rely on maternal diet and metabolic conditions. Our aim was to study the effects of maternal glycation during breastfeeding in offspring metabolism, neurodevelopment and behaviour.

Materials and Methods: Experimental groups (3): male offspring of lactating females treated with S-p-bromobenzylglutathione cyclopentyl diester (BBGC, 5mg/kg, day 1–6 of lactation) – selective inhibitor of glyoxalase-1 (maternal glycation); a control group; a vehicle group (treated with dimethyl sulfoxide). Between postnatal day (P) 5 and P17, offspring were subjected to neurodevelopmental tests. After weaning (P21) maternal milk was collected to measure triglycerides and total antioxidant capacity; at P43 offspring were tested in the Elevated Plus Maze and Open Field tests; at P45, the glycaemic profile and triglycerides were evaluated, and the hippocampus was collected.

Results: Maternal glycation reduced milk triglyceride levels and total antioxidant capacity, also impairing offspring body weight gain. No glycation-induced changes were detected in offspring triglycerides levels, whereas in insulin tolerance test, there was a significantly decay of glucose rate over the time, when compared with the control. In the offspring hippocampus, it was observed a higher content of advanced glycation end products, as well as decreased glyoxalase 1 levels and increased total insulin receptor levels. Maternal glycation impairs offspring vestibular and olfactive system development and anticipates offspring eye opening. At adolescence, maternal glycation induces a disinhibition and anxiolytic-like effects in the offspring, together with upregulation of

$GABA_a$ levels in hippocampus, which can underpin behavioural alterations.

Conclusions: The early exposure to glycation changes breastfeeding milk composition that are associated with offspring metabolic and neurodevelopment alterations, modulating its behaviour at adolescence.

56ASM-0220 | Profiling short-chain fatty acids (SCFA) producing microbiota in ulcerative colitis

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Background: Ulcerative Colitis (UC), an inflammatory bowel disease, is characterized by chronic inflammation of the colon, with severe and persistent mucosal inflammation and associated with an altered microbial composition.

Materials and Methods: Fecal microbiota composition was analyzed in 44 healthy donors and 10 UC patients by 16S rRNA gene sequencing.

Results: We have revealed significant shift in the spectrum of SCFA producing bacteria. Total amount of acetate producing bacteria has increased from 20,2% in the group of healthy subjects up to 50,3% in UC patients, mainly due the elevation of *Bacteroides* and *Streptococcus* spp. At the same time, there was a decrease in the amount of *Bifidobacterium* and *Ruminococcus* spp. A significant increase in propionate producing bacteria has been mainly associated with *Bacteroides* spp. and resulted in 28,8% of propionate producing bacteria in UC patients as compared to 7,59% in healthy donors. Butyrate deficiency in UC has been described multiple times, we have also found 7-fold decrease in the amount of butyrate producing bacteria in the UC patients: 3,0% in UC versus 22,4% for healthy subjects due to drop out of Faecalibacterium, *Bifidobacterium*, *Clostridium* and *Coprococcus* spp. Low level of *Coprococcus* and *Dialister* spp. found in the UC patients is also attributed to depression; decrease of *Bifidobacterium* spp. and as well of its product gamma-aminobutyrate acid could lead to anxiety, insomnia and stress reactions, often met in UC, providing strong evidence for gut-brain axis.

Conclusions: Altogether, these data indicate that disproportion of synthesized SCFAs may play key role in supporting inflammatory background and cause further disruption in the balance of microbial communities during the development of ulcerative colitis.

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56ASM-0236 | Potential of Côa Valley (Portugal) plants extracts in cell model for hepatic lipid toxicity

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Background: The World Heritage Site known as Foz Côa Valley (Portugal) is home to over 500 plants with medicinal properties. Some of those species show hepatoprotective effects. We tested extracts from two species in a lipid toxicity hepatic cell model exploring their potential therapeutic use for non-alcoholic fatty liver disease (NAFLD).

Materials and Methods: Human hepatocellular carcinoma HepG2 cells were grown in low-glucose media (DMEM with 10% FBS). The extracts tested were obtained from *Rumex scutatus* subsp. *induratus* (Boiss. & Reut) Nyman (infusion – RI-Inf, decoction – RI-Dec and 80% ethanol – RI-Et80) and from *Plantago coronopus* L. (80% ethanol – PC-Et80). The cytotoxicity effects were evaluated for 24h by measuring cell mass and metabolic viability. To induce lipotoxicity, 0.5mM of Palmitic Acid (PA, 0.5mM) and a mixture of Free Fatty Acids (FFA, 0.25mM and 0.5mM) were added to cells for 24h. The potential

protective effect of RI-Et80 was determined by quantifying metabolic viability, lipid accumulation, and cell mass. For lipoprotection studies, cells were incubated with different concentrations of Silibinin (MW: 482.44 g/mol, 50–600 μM - positive control) and the RI-Et80, 24h prior to PA and FFA addition.

Results: Cytotoxicity assays of extracts show that concentrations under 400mg/mL were non-toxic. The results show that the lowest concentrations of Silibinin (50 and 100 μM) and RI (50, 100 and 200 μg/mL) were effective in reducing lipid accumulation, especially following PA addition, without affecting metabolic viability and cell mass. Silibinin effects were confirmed by confocal microscopy using fluorescent dyes.

Conclusions: These promising results show potential protective effect of *Rumex scutatus* subsp. *induratus* in decreasing lipid accumulation on an *in vitro* cell model of lipotoxicity, with potential for follow-up studies in more complex NAFLD models.

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56ASM-0245 | Dietary recommendation in IBS: from empiricism to evidence

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Background and aim: Irritable bowel syndrome (IBS) has a true prevalence of 4% according to the Rome Global Study survey with Rome IV questionnaires. Being frequent, its management is very important. Diet is important in preventing IBS symptoms. We aimed to propose an evidence based corpus of recommendations for IBS patients.

Methods: 1. In a qualitative analysis, we identified in IBS patients of all subtypes, food that was considered by them to trigger symptoms or to relieve symptoms. 2. We elaborated a diet pyramid for IBS patients and a National Guideline for non-pharmacological therapy in IBS, both based on evidence.

Results: 1. Many patients make wrong attributions of aliments incriminating the occurrence of IBS symptoms and adopt empirically restrictive diets without scientific meaning. 2. The evidence-based method was able to identify healthy food to be recommended in IBS patients and also the role of probiotics, prebiotics, FODMAP, gluten, lactose free etc. diets in IBS. We present these two outcomes of evidence-based recommendations elaborated by two groups of specialists. The main feature of these outcomes is in fact the low level of evidence and the low degree of recommendations in IBS.

Conclusions: These results support the superiority of evidence-based recommendations to advice IBS patients in respect to their meals, versus the empirical recommendations. However, the degree of recommendations for these is low, explaining the difficulty of managing patients with IBS.

56ASM-0247 | The relationship between anxiety and depression in metabolic-dysfunction-associated fatty liver disease and associated cardiovascular outcomes

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Background: The link between anxiety and depression in metabolic-dysfunction-associated fatty liver disease (MAFLD) and cardiovascular (CV) risk is not well understood. Accordingly, we evaluated whether anxiety and depression are linked to structural and functional CV parameters.

Materials and Methods: A cross-sectional study (77 subjects -39 MAFLD patients and 38 controls), with hepatic steatosis evaluated using hepatic ultrasonography and SteatoTestTM, in addition to CV parameters using electrocardiogram (ECG), echocardiography and Doppler ultrasound was conducted. Anxiety (Lehrer Woolfolk Anxiety Symptom Questionnaire -LWASQ) and depression (Beck Depression Inventory -BDI) symptoms were assessed using self-report questionnaires.

Results: MAFLD patients had significantly higher BDI and LWASQ global scores than controls, with a p-value of 0.009 and 0.045, respectively. Significant ECG changes including prolonged p-wave (p-value = 0.002) and PR interval (p-value = 0.008) durations were observed in MAFLD patients, with non-significantly prolonged QTc (Bazett's

formula) (p-value = 0.064) in MAFLD patients. In linear analysis, the LWASQ somatic factor was significantly associated with global longitudinal strain (GLS) -0.0404, p-value = 0.002). However, this association was attenuated in multivariate analysis (-0.0166, p-value = 0.124). Although the group (MAFLD vs. controls) predicted the BDI, LWASQ global score, and LWASQ somatic factor in linear regression, they were no longer significant in multivariate analysis. Furthermore, the relationship between interventricular septal wall thickness (IVSWT) and BDI, LWASQ global score, and LWASQ somatic factor was significant in linear analysis, but lost significance after multivariate analysis.

Conclusions: Even though MAFLD patients were found to have an increased risk of anxiety and depression in univariate analysis, this correlation was attenuated to non-significant levels in multivariate analysis. MAFLD patients were found to have prolonged P-wave and PR interval durations. Moreover, in univariate analysis, we found a significant association between GLS levels and the LWASQ somatic factor, as well as the IVSWT, in anxiety and depression.

56ASM-0257 | Early alteration of liver function linked with liver stiffness and steatosis, but not with fibrosis

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Background: Non-alcoholic Fatty Liver Disease (NAFLD) is the most frequent chronic liver disease. Aim of the present study was to explore the relationships between Body Mass Index (BMI, Kg/m2), liver steatosis and/or fibrosis, and possible subclinical alterations of hepatic function assessed by orally-administered (13C)-methacetin and breath test (MBT).

Materials and Methods: A total of 60 consecutive subjects undergoing routine assessment for metabolic disorders (obesity and/or type 2 diabetes) entered the study. FibroScan® (EchoSens, Paris, France) served to assess the presence of liver steatosis (controlled attenuation parameter, CAP > 250 dB/m) and fibrosis (liver stiffness, LS > 7 kPa). MBT was used to quantify the efficiency of hepatic extraction from portal blood flow (DOB_{15}) and liver microsomal function ($cPDR_{30}$).

Results: CAP revealed steatosis in 50%, 63%, and 87% of normal weight, overweight and obese subjects, respectively. In none of these subgroups LS values indicated a significant degree of fibrosis (i.e., values < 7 kPa in all cases). DOB_{15}, the marker of liver extraction for methacetin, tended to decrease in overweight (19.3 ± 1.3‰) and decreased significantly in obese subjects (15.7 ± 1.4‰), as compared to normal weight subjects (24.0 ± 2.2 ‰, $P = 0.008$). This index (but not $cPDR_{30}$) was also lower in subjects with- (17.2 ± 10‰) than in those without steatosis (22.0 ± 2.1‰, $P = 0.01$). LS was higher in patients with- (5.3 ± 0.4 kPa) than in those without steatosis (3.8 ± 0.2 kPa, $P = 0.0004$), and DOB_{15} negatively correlated with both the extent of liver steatosis (R = -0.33; $P = 0.01$) and LS (R = -034, $P = 0.008$).

Conclusions: Obesity and liver steatosis are linked with a decreased efficiency of liver extraction from the portal flow. This finding is likely mainly linked with intracellular lipid accumulation per se, rather than to a significant degree of fibrosis.

56ASM-0262 | Prevalence of in-hospital infections and antimicrobial resistance in an internal medicine ward. A prospective study

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Background: In-hospital infections are frequent, delay discharges and increase health costs, also due to a progressive increase of antibiotic resistance. The real burden of infections and antimicrobial resistance in acute, general hospital settings is still unclear.

Materials and Methods: Enrolled were all patients admitted (all causes) from December 10, 2021 to March 30, 2022 in a division of Internal Medicine. Patients with clinical and/or instrumental evidence of bacterial infection were examined to characterize microbes and antimicrobial resistance.

Results: A total of 152 consecutive patients entered the survey. Overall, in-hospital infection prevalence in the examined period was 19%. On average, 1.4 cases of infections per day were diagnosed. The prevalence of infections increased with age, being the lowest in subjects with less than 30 years (0.7%), and the highest in those with more than 70 years (9.9%). Blood culture was positive in 65.5% of infected patients. Urinary tract was the most common site of infection, followed by skin (mainly pressure ulcers). The majority of cultures (72.4%) were positive for

Candida. Candida albicans was the main responsible species for Candida infections (41.1% of positive cultures). However, non-albicans Candida species (i.e., C.glabrata, Krusei, tropicalis, lusitaniae, parapsilosis) were also highly frequent (31% of positive cultures). The most frequent bacteria were Klebsiella pneumoniae (34.4%), and Pseudomonas Aeruginosa (31%). All patients with a positive culture received antimicrobial and/or antifungal therapy. A total of 61 antimicrobial resistances was detected, with the most frequent among beta-lactam (42.6% of total resistances), oxazolidinone (19.7%) and antifungal drugs (16%).

Conclusions: The prevalence of in-hospital infections in an acute hospital setting is high, particularly in elderly. Candida colonization/infection also due to non-albicans species is very frequent, probably due to immunodepression, increasing antimicrobial resistance and extensive antimicrobial use. This last finding represents an underrecognized, emerging threat, with relevant implications in term of in-hospital management and possible primary prevention measures.

56ASM-0263 | Mediterranean diet: food for changing the NAFLD world

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Poor dietary habits are greatly contributing to various metabolic disorders, posing enormous burdens to patients and healthcare systems. In this context, non-alcoholic fatty liver disease (NAFLD) is becoming one of the major health concerns worldwide, with a rising prevalence of about 25% in adult population. The cornerstone for NAFLD management relies on lifestyles modification, which includes, healthy eating, weight loss when needed, and physical activity. However, several studies show that restricted caloric intake alone is not enough for NAFLD treatment, and the composition of diet plays a crucial role. The Mediterranean diet (MD) is one promising dietary regimen for treatment of NAFLD. The common traits of MD have attracted global interest, and it is widely approached to reduce the incidence of several chronic diseases (i.e., cardiovascular disease, cancer, and obesity). Recently, MD has been recognized by the World Health Organisation (WHO) as an effective strategy for the prevention of chronic diseases. MD is characterized by a high intake of vegetables, fruits, whole grains, nuts, and legumes, with fish and white meat as a predominant animal protein source, moderate consumption of alcohol (usually red wine), and low intake of dairy products, red meat, and sweets. The main source of fat is extra-virgin olive oil (EVOO), along with nuts. The large dietary lipid content consists of monounsaturated fatty acids provided by extra-virgin olive oil. As a result, MD is helpful in decreasing liver fat accumulation along with decreasing the risk and severity of liver steatosis. Apulia (located in Southern Italy), where food intake perfectly matches the MD, has now reported intriguing data showing a decline in the adherence to MD, and a progressive increase in the prevalence of overweight and obese adults. Despite the increasing popularity of MD worldwide, more reeducational programs are required to promote MD and overall healthy lifestyle habits.

56ASM-0264 | Gastrointestinal diseases and gender

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Gastrointestinal (GI) diseases are among the most common problems which affect the general population and include a wide range of conditions, namely functional GI diseases, inflammatory diseases, celiac disease, and neoplasms. Although GI diseases affect people of all genders, the pathophysiology and the clinical manifestations vary depending on biological sex and gender role. Different incidences of specific GI diseases according to sex suggest a possible role of hormones in the disease development. Sex hormones might modulate GI motility, intestinal permeability, and barrier function, mucosal immunity, and intestinal microbiota. Moreover, psychosocial characteristics, gender-related differences in neuroendocrine, autonomic nervous system, and stress reactivity might impact bowel function and pain. Based on several evidence, it is mandatory to look at the differences in the development of GI diseases and treatment response according a perspective of "gender medicine" to improve clinical outcome.

56ASM-0269 | Cellular models of metabolic associated liver-gut disorders: beneficial effects of polyphenols from thymbra spicata

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Background: The gut-liver axis can be involved in different metabolic associated disorders such as Non-alcoholic fatty liver diseases (NAFLD). *Thymbra spicata*, one of the most popular herbs used in food and medicine in Lebanon, has been reported to exert beneficial effects on cellular and animal models. *In vitro* models recapitulate the complex process of gut integrity/damage and can provide clues to mechanisms of action in such a context.
Materials and Methods: We developed different *in vitro* models of metabolic associated disorders to mimic different aspects of gut-liver axis disorders. We used cellular models for hepatic steatosis (lipid-loaded FaO cells), liver inflammation (LPS-challenged Kupffer cells), endothelial dysfunction (H_2O_2-challenged HECV cells) and gut dysbiosis (differentiated CaCo2). *T. spicata* ethanolic extract (TE) and carvacrol (CVL), the most abundant bioactive compound in TE, were tested on those models. Lipid accumulation, ROS production, inflammatory markers, and intestinal membrane integrity were measured by different colorimetric and fluorescent assays, molecular tests such as Western Blot and qPCR, and Ussing chamber.
Results: Hepatocytes overloaded with FFAs, damaged endothelium, and Kupffer cells exposed to LPS from dysbiosis gut represent a reliable *in vitro* model of gut-liver axis and NAFLD/NASH progression. TE, which is rich in polyphenols, and Carvacrol remarkably ameliorated hepatic steatosis by reducing lipid accumulation and fat-induced oxidative stress. In addition, TE ameliorated also the LPS-insulted inflammation in Kupffer cells, by decreasing a panel of inflammatory markers such as NF-kB, iNOS, TNFα. On CaCo2 cells, TE exerted cytoprotective effects and modulated intestinal integrity.
Conclusions: Our models recapitulate gut absorption of fatty acids, LPS leakage, lipid accumulation and inflammation in liver cells. *Thymbra spicata* and CVL exert an interesting lipid-lowering, anti-inflammatory, and antioxidant ability at difference cellular levels. The role of these nutraceutical agents to counteract NAFLD/NASH progression and to modulate Gut-Liver crosstalk is actively being investigated.

56ASM-0272 | Mediterranean diet adherence and metabolic syndrome in lebanese population: a real-time survey study

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Background: Mediterranean Diet (MD) provides one of the healthiest available dietary habits to lower the risk of cardio-metabolic diseases. The relationship between adherence to Mediterranean diet and prevalence of metabolic related conditions such as overweight/obesity, and non-alcoholic (metabolic associated) fatty liver disease (NAFLD/MAFLD) has been investigated by several epidemiological and clinical studies. However, no study explored the interaction between MD and metabolic syndrome (MetS) in Lebanon.
Materials and Methods: We designed a google-form survey between July 2021 and March 2022 in free-living Lebanese people to explore lifestyles and diet, MetS, and the presence/stage of NAFLD/MAFLD. A validated score for adherence to the Mediterranean diet (MD score ranging from 0 = no adherence to 18 = highest adherence) was calculated for each participant. The presence of MetS was recorded based on standard ATPIII criteria.
Results: A total of 731 participants (age 32.6 ± 11.8 years, 476 females) answered the questionnaire. We categorized groups based on BMI as normal weight (369; 50.5%, F:M = 277:92), overweight (241;33%, F:M = 137:104) and obese (121;16.5%, F:M = 62:59). Overall, the MetS was present in 22 subjects (3%), i.e., 14%, 2%, 0% in obese, overweight, and normal weight subjects, respectively. NAFLD/MAFLD was reported by 32 (4.4 %) subjects, i.e., 15%, 4% and 1.1% in obese, overweight and normal weight subjects with prevalence for men in comparison with women (F:M = 10:22; 2.1% vs 8.6%). Interestingly, the MD score was inversely correlated with BMI, being significantly ($p < 0.05$, ANOVA) higher in normal weight (9.07 ± 2.3) than overweight (8.4 ± 2.1) and Obese (7.28 ± 2.2) subjects.
Conclusions: The awareness of both MetS and NAFLD/MAFLD is likely underestimated in Lebanon, as confirmed by low prevalence rate reported by self-administered questionnaire. With this limitation in mind, we found an inverse association between Mediterranean diet adherence and obesity-related MetS in Lebanon.

56ASM-0273 | Post-Long)-COVID: The burden in internal medicine. a never-ending story

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Background: Persisting or novel symptoms are frequent following recovery from acute COVID-19. Aim of the present study was to explore symptoms and signs detectable up to 1 year following a SARS-Cov-2 infection.

Materials and Methods: In a dedicated post-COVID-19 outpatient clinic we enrolled, since December 2020, 432 patients (184 females, mean age 49 ± 14SD years) with two negative and consecutivenasopharyngeal swabs following a COVID-19 diagnosis. A total of 114, 150, 157 and 25 subjects were also observed after 1, 3, 6, and 12 months from recovery. The previous infection was mild (home quarantine) in 344 patients, whereas 88 subjects underwent hospitalization. A questionnaire served to explore the presence of specific symptoms (fever, dyspnea, fatigue, arthro-myalgia,chest pain, cough, anosmia, headache, sicca syndrome, rhinitis, dysgeusia, alopecia, diarrhea, loss of appetite, dizziness, sweating, anxiety/depression, memory impairment, insomnia, concentration impairment).

Results: The prevalence of the following six symptoms progressively decreased from baseline to 3 months: fatigue (from 68% to 37% $P < 0{,}0001$), fever (from 59% to 0%, $P < 0{,}0001$) arthro-myalgia (from 55% to 25%, $P < 0{,}0001$), cough (from 54% to 13%, $P < 0{,}0001$), dysgeusia (from 46% to 7%, $P < 0{,}0001$), dyspnea (from 45% to 22%, $P < 0{,}0001$). Conversely, memory impairment (from 15% to 33% $P < 0{,}0001$) and concentration impairment (from 20% to 33% $P = 0{,}0014$) increased from baseline to 3 months. At 6 mo., memory and concentration impairment, and anxiety/depression persisted in 25-30% of patients. At 12 mo., 40%, 32% and 28% of patients still reported fatigue, impaired memory and/or concentration, respectively.

Conclusions: Long-lasting post-COVID symptoms can be present also following a mild COVID infection. While the prevalence of the majority of symptoms progressively decrease in about 6 months, fatigue and neuropsychological symptoms can persist up to 12 months, indicating the need for long-term follow-up and adequate clinical management.

56ASM-0276 | Small intestine derived extracellular vesicle mediators in the gut-liver axis crosstalk

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Background: Diabetes and obesity are widely recognized metabolic disorders of the 21st century largely due to sedentarism and caloric-unbalanced diets. Inter-organ communication is central for metabolic homeostasis. Recently, one form of organismal communication captured our attention: the extracellular vesicles (EVs), which are proficient in triggering functional alterations in recipient cells by realising their cargo. Gut dysbiosis is emerging as a diabetogenic factor. In fact, metabolic surgery is the only intervention in which diabetes remission occurs. Suggesting that the gut plays an important role in the etiology of diabetes. We unveiled that gut-derived EVs' (GDE) protein content reflects the metabolic state of the organism. Therefore, we hypothesize that GDE are mediators of gut-liver axis in type 2 diabetes pathogenesis and progression.

Materials and Methods: Two groups of mice were fed with control diet or high fat diet (HFD) for 12 weeks. Upon confirmation of prediabetic phenotype, EVs were isolated by ultracentrifugation. EVs lipidic content was profiled. Labeled GDEs were retro orbital injected into normoglycemic animals for 6 weeks and their biological impact was studied.

Results: Chronic exposure of healthy mice to GDE revealed that GDE have affinity to the liver and are preferentially uptaken by the resident macrophages of this tissue, the Kupffer cells. We observed that mice which received HFD-GDE had significantly higher weight gain, in comparison with the control. Interestingly, there was a

positive correlation between the glucose tolerance phenotype of donor mice with the glucose tolerance of the correspondent recipient mice, i.e. the animal injected with GDE from that donor. Moreover, mice which received HFD-GDEs manifested increased levels of hepatic triglycerides. These evidences further strength that the altered protein and lipidic content in HFD-GDE are translated into hepatic biological alternation.

Conclusions: In total, we demonstrated that GDEs are vehicles of communication in the enterohepatic axis, and disclosed lipids and proteins as their relevant cargos.

56ASM-0290 | Monitoring palatability and gastrointestinal symptoms after acute and long-term consumption of four types of gluten-free pasta in healthy subjects

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Background: Functional foods have a positive impact on metabolic profiles. Assembling innovative "Italian" pasta combined with alternative wheat (legume) flours represents a challenge for the food industry and can provide beneficial effects on health. Innovative foods, however, deserve careful assessment, including the ultimate influence on taste and compliance.

Methods: Forty healthy volunteers consumed in a long-term (30 days), four different and innovative types of pasta (80 g pasta, 13 g olive oil, $N = 10$ subjects/group) containing either red lentils, green beans, buckwheat or rice/corn (Andriani SPA-Natural innovators for conscious food, Gravina in Puglia, Italy). At baseline and at the end of the study, semi-quantitative questionnaires were administered to monitor the appearance of upper gastrointestinal symptoms as severity, frequency, and duration. The pleasantness and digestibility of pasta were evaluated by visual analogue scales.

Results: At baseline, the palatability of the 4 different types of pasta was overall high, but higher in legume-based (lentils 64.5 ± SEM4.1 mm; beans 70 ± 5.2 mm) and rice/corn pasta (51 ± 8 mm) than buckwheat (26 ± 3.7 mm, $P = 0.00001$). Bean pasta caused a greater gastric fullness (Area Under Curve) than buckwheat paste (393.2 ± 42.8 mm } 120 min vs 152.2 ± 48.7 mm } 120 min, $P = 0.027$). Rice/corn pasta seems tended to cause a more intense abdominal bloating than other types of pasta. At the end of the follow-up (day 30), postprandial fullness decreased in consumers of buckwheat and rice/corn pasta. Pleasantness and digestibility remained elevated in all types of pasta.

Conclusions: Appropriate monitoring of palatability and gastrointestinal symptoms of "healthy" foods like types of pasta provides accurate information on the overall compliance of target consumers. Pasta added with alternative flours is generally well tolerated. Studies are in progress to extend the study of different types of pasta on gastrointestinal motility, metabolic indices, and gut microbiota adaptation.

56ASM-0291 | Different variations of intra-familial body mass index subjected to COVID-19 lockdown

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Background: The COVID-19 lockdown has represented an inedited model of increased metabolic risk in all age groups, due to negative changes in dietary habits, physical activity, lifestyle. These effects have been generally explored at a population level in distinct age groups. Potential intra-familial, specific effects in adults and children sharing the same socio-economic, cultural level and living habits have been scarcely explored. We aimed to characterize changes of anthropometric indices in parents and in their children during lockdown.

Methods: a cohort of 149 couple parent/children were prospectively enrolled. By a validated questionnaire we explored changes of Body Mass Index (BMI) and individual lifestyle during a 2-month lockdown (May-July 2020).

Results: BMI increased in 70.5% of parents and in 67.8% of their children, with a D-BMI of 1.44 + 0.09 kg/m^2 and

0.36 + 0.02 Kg/m^2, respectively. BMI increments, however, were only significant in adults and did not correlate in the couple parents/children. Most adults (80.5%) and children (71.4%) did not perform regular physical activity during the lockdown. Direct correlations between dietary changes and BMI variations became evident in children, mainly in terms of decreased consumption of fresh fruit, pulses, fish, and increased consumption of cereals, carbohydrates, dairy products, olive oil. In normal weight, overweight and obese children, but not in adults, the increase in sleep hours increased with BMI.

Conclusions: despite marked lifestyle changes imposed by the lockdown, BMI variations in parents were independent from those observed in their children, pointing to different outcomes in response to the same external, critical event. Thus, primary prevention measures aimed at maintaining a healthy lifestyle require different approaches according to age

56ASM-0292 | A real-life survey in symptomatic COVID-19 patients taking medicinal herbs. Emerging role for *Za'atar* on the background of complementary and alternative medicine

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Background: The burden of the COVID-19 pandemic worldwide has led to the search for appropriate treatment for acute infection, besides preventive vaccines. Despite scientific evidence on the effects of herbs on SARS-COV-2 is lacking, complementary and alternative medicine (CAM) has broad popularity and appeal in Lebanon and includes medicinal plants. One issue is the popular interest in medicinal plants for prevention or treatment of respiratory symptoms during SARS-COV-2 infection.

Methods: From April-June 2021 we designed an online anonymous questionnaire (26 items) to investigate many aspects related to 1st-2nd wave COVID-19 pandemic in Lebanon. We explored the presence and severity of COVID-19 symptoms, the use, and putative effects of popular herbal products. A symptom score was calculated by summing the intensity (0 = min to 5 = max) of 13 different symptoms (max score = 65).

Results: A total of 557 subjects (age 1–80 years, 233 males) answered the questionnaire (including parents of minors). The most frequent reported symptoms were fatigue (88.8 % of cases), headache (83.5%), and anosmia (80.4 %). Post-COVID symptoms were reported by 49% of the patients. Aspects linked with depression, anxiety, and fear during and/or after COVID-19 were reported by 31.5% of participants. Most subjects (60.5%) believed that herbs "*can cure or prevent diseases*" and 55% used herbal products during the acute phase of infection. The mixture *Zhourat* (32%), *Za'atar* (9.3%), Anise (8%) and Ginger (9.2%) were the most popular herbs, with 60% of participants reporting the decreased intensity of some symptoms. *Za'atar* yielded the best outcome (symptom score 17.2 ± 13 *vs* no herbs 23.2 ± 13.3, Anise 28.7 ± 12.0, *Zhourat* 25.3 ± 12.5, and Ginger 23.6 ± 12.3; $P = 0.0005$).

Conclusions: Preliminary data from this open survey finds that according to the popular perception, *Za'atar* brings more pronounced beneficial effects than other commonly employed herbal products, both during the acute phase of the disease and in the post-COVID-19 period. *Za'atar* is enriched with bioactive compounds such as polyphenols with protective effects against oxidative stress and inflammation, two major pathogenic mechanisms involved in determining the severity of COVID-19. The results from this survey urge methodological confirmation by controlled trials.

56ASM-0293 | Cell therapies in Crohn's disease

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Crohn's disease (CD) is a lifelong segmental inflammatory enteropathy caused by a dysregulated immune response towards bacterial antigens that develops in genetically susceptible individuals. Three behaviors are recognized, i.e., inflammatory, structuring and penetrating, with the last one being the most invalidating. The advent of cellular therapies, mainly based on the use of mesenchymal stem/stromal cells (MSCs) represents a great step forward thanks to their immune-evasiveness, immunomodulatory effect and high safety profile. Recently, several phase 1–2 studies were carried out, where the use of autologous or allogeneic systemic infusions of bone marrow- or placenta-derived MSCs for treatment-resistant inflammatory CD was tested. These studies showed that this therapeutic approach is feasible and safe, as well as significantly effective since disease remission was achieved in half the patients despite only refractory cases were enrolled. Remarkably,

the best outcome was obtained when serial infusions were performed. This opens up the question of the half-life or at least the duration of the therapeutic effects of MSCs, a crucial point in establishing the right timing for infusions. Clearer and more unambiguous results were obtained when using MSC local injections for fistulising refractory Crohn's disease. Notably, the results of the first phase III multicentre trial, where 212 patients were enrolled and randomly assigned to receive a single local injection of an industrial preparation of allogeneic adipose tissue derived-MSCs (Darvadstrocel®) or placebo, showed that MSCs performed better than placebo to achieve remission at week 24 within a shorter period of time. Moreover, fistula healing was maintained in most cases at one year, although further studies showed that the proportion of patients relapsing upon a longer follow-up increased over time. An *in vitro* study showed the ability of MSCs to induce apoptosis of mucosal T-cells isolated from CD patients and to decrease pro-inflammatory cytokines, thus interrupting the magnification of inflammation, while a sustained increase of regulatory T-cells was observed *in vivo*. Finally, MSCs were also successfully applied in an experimental model of colonic fibrosis where the ability to inhibit the accumulation of fibrotic tissue, the expression of fibrotic molecules and the epithelial-to-mesenchymal transition was clearly evident. Should these results be confirmed in clinical trials, the chance of safely and efficaciously treating this dreadful condition will become a real prospect.

56ASM-0301 | The role of quantitative ultrasonography as tool to monitor the characteristic visceral fat accumulation in psychiatric patients on atypical antipsychotic

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Background: Psychiatric patients have a significantly increased risk of morbidity and mortality due to multiple factors including metabolic alterations of visceral fat accumulation secondary to therapies with atypical antipsychotics. A quantitative assessment of subcutaneous and visceral body fat can provide information to better monitor the outcome of therapeutic strategies.

Methods: We enrolled 28 psychiatric patients (PP) (0 lean, 11 overweight, 17 obese) on atypical antipsychotics (range 0.2–25 yrs) and 132 controls (CO) (48 lean, 44 overweight, 40 obese). By semi-quantitative/quantitative abdominal ultrasonography (*Noblus* Hitachi, Eurisko, IT), we assessed the presence and severity of nonalcoholic fatty liver disease (NAFLD). In addition, we exactly measured abdominal fat in four subcutaneous districts, i.e., anteriorly to left liver lobe of the liver, anterior to splenic vein, preaortic region, nuchal fat, and in five visceral districts i.e., anterior to left liver lobe, anterior to splenic vein, preaortic region, hepatorenal fat, splenorenal fat.

Results: PP and CO had comparable age (42 ± SD13 yrs and 44 ± 13, resp.), but increased BMI (32.5 ± 0.9 vs 27 ± 0.4 Kg/m2, resp., $P < 0.0001$), increased obesity rate (60.1% vs. 30.0%, resp., $P < 0.005$), and NAFLD rate (64% vs. 43% resp. $P < 0.05$). Subcutaneous fat was similar in PP and CO (range 13–19 mm, P = NS). PP had increased visceral fat thickness in 2 out of 5 districts, i.e., anterior to left liver lobe (respectively 15.2 ± 1.0 vs. 10.5 ± 0.5mm; anterior to splenic vein 52 ± 3.4 vs. 33 ± 1.3mm, $P < 0.001$). In addition, visceral fat anterior to the splenic vein was increased in overweight PP compared to overweight CO (45.5 ± 5 mm and 32.7 ± 1.6 resp., $P = 0.003$) and in obese PP compared to obese CO (57.6 ± 4.4mm in PP vs. 43 ± 2.6mm in C, resp., $P < 0.005$).

Conclusions: PP on atypical antipsychotic drugs show increased early obesity rate and exaggerated visceral fat thickness compared to age-matched controls. The finding persists in overweight/obese subgroups of PP when compared to matched controls. The supplemental burden of visceral fat in PP on antipsychotic drugs represents a useful marker of metabolic risk in these patients.

56ASM-0303 | Mediterranean diet in developmental age: current evidence and research gaps

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Background: There is evidence that the Mediterranean diet (MD) can reduce the risk of developing obesity in pediatric patients during developmental age. Both physical changes and environmental/social influences, however, can influence specific age phases and evidence require careful assessment.

Methods: We conducted a literature search in PubMed, Web of Science, Google Scholar, and Scopus of published

articles between 1st January 2005 and March 2022 on MD across developmental ages from fetal development, breastfeeding and weaning, preschool- and school childhood age, and adolescence.

Results: A total of 117 clinical studies were grouped according to the following keywords: "Mediterranean Diet" and "Pregnancy"; "Breastfeeding"; "Weaning"; "Child"; "Pre-school age"; "School-age"; "Adolescence". After excluding non-English papers, case reports, conference abstracts, and studies considering only general aspects, we analyzed 58 articles. We found that the evidence was lacking about MD adherence in age groups between 0.5–1 year of life (during which weaning takes place) and parents. Although the issue of adherence to the MD and its beneficial effects on obesity prevention were extensively investigated in the other age groups, few studies have addressed the role of MD lifestyle as a strategy to decrease the risk of other non-communicable diseases.

Conclusions: By analyzing current literature, we conclude that adherence to MD must be encouraged during the developmental age. Research on the multi-intervention strategy must be implemented, to promote the education of children and families by emphasizing changes toward healthy lifestyles. Highlighting the gaps of specific information for each age group that can provide new research perspectives.

56ASM-0304 | Clinical association between visceral fat and risk of type 2 diabetes

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Background: In the last decades, enormous clinical evidence reported that obesity, as a general term, represents a risk factor for type 2 diabetes (T2D). However, a distinct association of T2D with visceral adipose tissue (VAT) phenotype is less clarified. Herein, we aimed to analyze clinical studies on the molecular and phenotypical impact of VAT on T2D.

Methods: PubMed, Web of Science, Google Scholar, and Scopus were searched for full-text papers in English published from 2005 to March 2022. We focused on clinical studies reporting the association between VAT and T2D. Case reports, conference abstracts, and studies considering only general obesity were excluded.

Results: A total of 204 clinical studies matched our keyword search. Twenty-five observational and interventional clinical studies met the inclusion criteria. Data recapitulate around 500 diabetic subjects undergoing VAT measurement by different methods. Our analysed data revealed that, unlike BMI and general obesity, VAT measurement was a stronger and more accurate predictor of body fat content. Consistent studies report that higher VAT expansion is closely associated with an increased risk of T2D. At the molecular level, many studies reported that VAT content increases with HbA1c while insulin sensitivity decreases.

Conclusions: Recent evidence highlights the correlation between VAT and T2D risk. In addition, many interventional studies using diet, lifestyle, or drugs have chosen VAT expression as a biomarker and risk factor in T2D populations. Our ongoing research is focusing on the clinical and molecular markers of metabolic risk profile including accurate assessment of VAT expansion and function in T2D patients with respect to additional risk factors as components or fellow travellers with the metabolic syndrome (i.e., gallstone disease, liver steatosis).

56ASM-0307 | Sucrase isomaltase variants and their role in irritable bowel syndrome

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Irritable bowel syndrome (IBS) affects a large fraction of the general population with chronic symptoms including constipation (IBS-C), diarrhea (IBS-D), bloating and abdominal pain. Etiology is mostly unknown, and the pathophysiology likely involves several factors including genetic predisposition and dietary risk factors. Sucrase-isomaltase deficiency is a form of carbohydrate malabsorption characterised by clinical symptoms similar to IBS-D. CSID results from defective glucosidase (disaccharidase) activity of mutant sucrase-isomaltase (SI) enzyme in the small intestine, leading to colonic accumulation of undigested carbohydrates and their bacterial fermentation. We recently reported CSID cases misdiagnosed as IBS, and also showed that common SI dysfunctional (hypomorphic) polymorphisms associate with increased risk of IBS in multiple tertiary centre case-control cohorts. Most recently, in a large-scale survey of more than 180,000 individuals from the UK Biobank, we also detected higher prevalence of SI hypomorphic variants in participants with a diagnosis of IBS in their medical records compared to the rest of the population, thus providing compelling evidence also at the level of the general population. Finally, we propose that SI carrier status may be relevant to the efficacy of dietary interventions, since SI carriers were less likely to benefit from a low-FODMAP diet in a

pilot retrospective study of IBS-D cases. Our studies suggest that a fraction of IBS patients may experience symptoms because of defects in the digestion of carbohydrates due to hypomorphic variants in the SI gene and, possibly, other similar genes. This is likely to be relevant to personalising the treatment in a subgroup of patients.

56ASM-0307 | Neuro-immune signaling in the gut

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The gut-brain-axis is represented by the neural connectivity linking the gut, with its intrinsic innervation, i.e. the enteric nervous system (ENS), to the brain. The connection is anatomically provided by other two extrinsic components of the autonomic nervous system, the parasympathetic and the sympathetic innervation, conveying a wide array of stimuli (mainly of sensory origin) to central nuclei and from there to the cortex where the integration generates sensations. Most of these stimuli derive from the gastrointestinal (GI) tract, where a myriad of immune cells are distributed in a close relationship with the nerve processes of the ENS and other extrinsic neural components. This microanatomic paradigm recognizes the mast cells as one of the key cellular elements and supports consistent evidence indicating that an immune/mast cell-nerve signaling contributes to gut functions in health and disease states. A variety of morphological and imaging techniques are now available to assess structural and functional relationships of the mast cell-nerve signaling in human gut tissues revealing a prominent role in various functional GI clinical phenotypes ranging from irritable bowel syndrome to severe gut failure (e.g. intestinal pseudo-obstruction syndrome). Several factors account for this variability in clinical expression: first, etiological agents (e.g., infectious); second, patient's genetic background; third, the predominant localization of the immune response (mucosal innervation *vs*. enteric ganglia of the ENS); fourth, mast cell mediators involved; fifth, the duration of the disease along with the existence of comorbid conditions (e.g., psychiatric disorders). This knowledge represents the framework to better understand how to control immune/mast cell-neural signaling in functional bowel disorders as well as in severe GI sensorymotor dysfunction.

SYMPOSIUM 2: MITOCHONDRIA

56ASM-0237 S2 IS | Keeping mitochondria in shape: a matter of cell life and death

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Background: In the last years, mitochondrial ultrastructural and morphological changes have been implied in the control of several physiological and pathological changes, including the progression of apoptosis, inflammation, differentiation, tumorigenesis.
Materials and Methods: However, the role of mitochondrial dynamics in the control of complex cellular cues and in response to reversible and irreversible cellular damage is not yet clarified.
Results: We will overview the key experiments that shed light on the role of mitochondrial shape and ultrastructure in cell physiology, pathology and in disease.
Conclusions: We offer a personal perspective on the missing pieces of the puzzle that await to be studied.

56ASM-0162 S2 IS | The mitochondrial permeability transition: recent progress and open questions

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Major progress has been made in defining the basis of the mitochondrial permeability transition, a Ca^{2+}-dependent permeability increase of the inner membrane that has puzzled mitochondrial research for almost 70 years. Initially considered an artifact of limited biological interest by most, over the years the permeability transition has raised to the status of regulator of mitochondrial ion homeostasis and of druggable effector mechanism of cell death. The permeability transition is mediated by opening of channel(s) modulated by matrix cyclophilin D, the permeability transition pore(s) (PTP). The field has received new impulse (i) from the hypothesis that the PTP may originate from a Ca^{2+}-dependent conformational change of F-ATP synthase; and (ii) from the re-evaluation of the long-standing hypothesis that it originates from the adenine nucleotide translocator (ANT). I will provide an account of how ANT and F-ATP synthase may form high-conductance channels, and discuss how unraveling the molecular components of the PTP will allow a reassessment of the role of the permeability transition in cell death.

56ASM-0281 S2 IS | Mitochondria and lysosomes at the helm of cellular metabolism

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Mitochondria and lysosomes were long considered as independent entities in the cell. Recently, evidence was reported that defects in one of these organelles result in perturbations of the other. Nevertheless, the mechanisms remain unclear.

Using mouse models of mitochondrial and lysosomal disease, we have unveiled a bi-directional communication pathway between mitochondria and lysosomes. Mitochondrial dysfunction results in lysosomal saturation and impairment of lysosomal calcium signaling and accumulation of lysosomal substrates, including autophagy intermediates. On the other hand, the impairment of lysosomes results in mitochondrial malfunction via impairment on the homeostasis of iron. Furthermore, mitochondrial and lysosomal dysfunctions have opposite effects on lipid metabolism. We will integrate these findings into an overall picture of organelle homeostasis in physiology and disease.

56ASM-0238 S2 IS | The importance of interactions between the er and mitochondria in determining cell fate

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Mitochondria are the organelles that fulfil a number of key functions in the cell, from energy supply, through metabolism regulation to the control of the cell death. Interactions of mitochondria with other organelles are crucial for the correct transfer of signals regulating multiple physiological processes and for the proper response to changes in the cellular environment, but also for the mitochondria themselves to enable their physiological functions. Membranes interacting physically and functionally with the outer mitochondrial membrane (mitochondria associated membranes, MAM), play a special role in this network of subcellular communication. Currently, its role is intensively studied in such processes as the regulation of energy metabolism, calcium homeostasis, redox balance, autophagy and apoptosis. Mitochondria – ER contact sites have been also shown to be crucial for the immune responses. Contact sites between the mitochondria and ER create a niche endowed with a set of characteristic proteins, which can be either permanently present, or temporarily transported into MAM to perform a specific function under certain conditions, e.g. oxidative stress. Many proteins involved in mitochondria – ER contact sites organisation have been linked to the oncogenic process and to the resistance of cancer cells to chemotherapeutics. For these reasons, mitochondria associated membranes are currently investigated in the context of many pathologies, including cancer, metabolic and neurodegenerative diseases. My particular interest is focused on the p66Shc protein and its interactions with other proteins in the MAM fraction. It has been demonstrated that these interactions are important for the cell's response to oxidative stress and apoptosis initiation. Such knowledge about the cell's fate determining proteins located in MAM fraction may contribute to the better understanding of the mechanisms controlling the transition from health to the disease.

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56ASM-0035 S2 IS | Assessing mitochondrial substrate utilization by 13C-enriched substrates and 13C NMR isotopomer analysis

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Background: Substrate oxidation by the Krebs cycle is the major pathway for the generation of reducing equivalents for oxidative phosphorylation in most cell types of the body. The choice of substrates that are oxidized can be influenced by several factors including a) overall availability of the substrate and its transport into cells and mitochondria and b), regulation of substrate catabolism at the metabolic pathway level via strategic enzymes such as pyruvate dehydrogenase. Meanwhile, the yield of reducing equivalents and/or ATP per mol of substrate oxidized is dependent on functional electron transport and oxidative phosphorylation by the mitochondria. Since the pathophysiology of many diseases may involve alterations in substrate and oxygen availability and/or mitochondrial dysfunction, a holistic analysis of substrate oxidation fluxes and mitochondrial function should provide powerful insights into disease progression and management. The objective of this presentation is to describe a powerful method for evaluating Krebs cycle substrate selection based on ^{13}C-NMR analysis of Krebs cycle isotopomers

generated from ^{13}C-enriched substrates and provide examples from our previous work where this approach was applied.

56ASM-0224 S2 IS | Bioenergetic cluster analysis – Diagnostic evaluation of mitochondrial respiratory control in human fibroblasts

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The medical community and our society are becoming increasingly aware of the role of mitochondria in pharmacology, diseases, and healthy aging. In contrast, mitochondrial diagnostics remain largely neglected in clinical trials: diagnostic services fail to support large- and small-scale projects and pilot studies. Quality control — necessary for clinical trials and highly deserved in any scientific study to ensure reproducibility [1] — is hardly affordable in the typical research laboratory [2].

In a meta-analysis of human skin fibroblasts, high-resolution respirometry and polarography covering cell senescence and the human age range were compared with multiwell respirometry to evaluate published data on mitochondrial function in relation to clinical diagnostic standards [3]. The common coupling control protocol measures ROUTINE respiration of living cells followed by sequential titrations of oligomycin, uncoupler, and inhibitors of electron transfer [4].

Bioenergetic cluster analysis BCA increases the resolution of outliers. *Isolinear* clusters are separated by variations in quantities that correlate with rates, whereas *heterolinear* clusters fall on different regression lines. *Dispersed* clusters are separated by critical threshold values. BCA provides guidelines for establishing quality control for bioenergetic databases in mitochondrial physiology. The present example on human fibroblasts as models of diseases may serve as a reference for bioenergetic mitochondrial diagnostics complementary to genomic or proteomic standards.

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56ASM-0277 S2 IS | Assessment of mitochondrial fitness in human blood

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Mitochondrial fitness is a prerequisite for cellular activity and homeostasis. On the contrary, mitochondrial dysfunction is a most prominent feature in human disease but also occurs upon negative environmental impacts. While both mitochondrial fitness and damage may primarily affect specific tissues, we nevertheless asked whether an overall systemic but actual status of mitochondrial fitness can be assessed.

We therefore developed means to assess mitochondrial bioenergetics in platelets from human but also rodent blood using high-resolution respirometry. While one platelet does only contain single figure amounts of mitochondria, due to their sheer abundance, they represent a quantitative mitochondrial key reservoir in blood. Moreover, as platelet life time is around ten days, they may give a most accurate picture of systemic mitochondrial fitness at a given time point of analysis. Importantly, platelets are directly accessible and metabolically highly active as they use both glycolysis and oxidative phosphorylation to cover their immense energy demand.

Our first results from human samples indicate that such platelet mitochondrial activity can be routinely and reproducibly assessed. Furthermore, preliminary data indicate that lifestyle changes but also human disease do impact on platelet mitochondrial fitness.

56ASM-0063 S2 IS | KRAS-regulated glutamine metabolism requires mitochondrial uncoupling protein 2-mediated aspartate transport to support pancreatic cancer growth

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Background: Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a very low survival rate. PDAC survival and proliferation rely on rewired glutamine metabolism induced by mutations in the human v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS). KRAS is the most frequently mutated gene in PDAC (up to 93%), which is the predominant form of pancreatic cancer (~90%). Oncogenic KRAS induces rewiring of the pentose phosphate pathway by decoupling ribose biogenesis from NADPH biosynthesis. KRAS shifts most of the glutamine-derived glutamate to the mitochondrial synthesis of aspartate in order to fulfil NADPH requirement for reductive biosynthesis and redox homeostasis. Aspartate, once transported to the cytosol, is converted through a series of reactions to pyruvate with the production of NADPH. The mitochondrial transporter responsible for this aspartate efflux has remained elusive.
Materials and Methods: UCP2 shRNAs were delivered in Patu8988T, Panc1, BxPC3 and KP2 PDAC cell lines by lentiviral transduction. Silencing was induced by doxycycline. The proliferation rate, clonogenic assay and $NADPH/NADP^+$ and GSH/GSSG ratios were determined with standard kits. Metabolomics analysis was carried out with [U-^{13}C]glutamine, mitochondrial and cytosolic pools were separated by digitonin fractionation.
Results: Here, we show that mitochondrial uncoupling protein 2 (UCP2) catalyses this aspartate transport and promotes tumour growth. UCP2-silenced $KRAS^{MUT}$ cell lines display decreased glutaminolysis, lower $NADPH/NADP^+$ and glutathione/glutathione disulfide ratios and higher reactive oxygen species levels compared to wild-type cells. UCP2 silencing reduces glutaminolysis also in $KRAS^{WT}$ PDAC cells but does not affect their proliferation rates or redox homeostasis. In vitro and in vivo, UCP2 silencing strongly suppresses $KRAS^{MUT}$ PDAC cell growth.
Conclusions: Collectively, these results demonstrate that UCP2 plays a vital role in PDAC, since its aspartate transport activity connects the mitochondrial and cytosolic reactions necessary for $KRAS^{MUT}$ rewired glutamine metabolism, and thus it should be considered a key metabolic target for the treatment of this refractory tumour.

6ASM-0202 S2 IS | | Evidence of a role for interleukin-6 in anoikis resistance and bioenergetic programming in oral squamous cell carcinoma?

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Background: Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide. In an endeavour to understand metastasis from oral squamous cell carcinomas, we characterised the metastatic potential (*anoikis* resistance) and bioenergetic profile of a human tongue derived cell line (SCC-4 cells) and compared this phenotype to pre-cancerous dysplastic oral keratinocyte (DOK) cells derived from human tongue.
Materials and Methods: Dysplastic DOK and cancerous SCC4 cells were cultured as instructed by the ECACC. FACS detection of propidium iodide and annexin-V were used to determine apoptosis/necrosis. ELISAs were used to determine cytokine levels. Immunoblots were used to determine key proteins/receptors. Poly(hydroxyethyl methacrylic) acid (poly-HEMA) coated plates, in combination with the apoptosis assay, were used to determine *anoikis* resistance. An Oroboros Respirometer and Seahorse Flux Analyzer were used to determine oxygen consumption rates. A Biolog TM mitoplate was used to determine a bioenergetic enzymic profile. Cell migration was determine by the "scratch assay".
Results: We demonstrate that SCC-4 cells constitutively synthesize and release significant amounts of IL-6, a process that is enhanced by the addition of the TLR2/TLR6 agonist, Pam2CSK4. The expression of TLR2/6 and IL-6Ra/gp130 receptors was also confirmed in SCC-4 cells. Cancerous SCC-4 human tongue cells also have a classic endothelial-mesenchymal transition (EMT) profile, unlike precancerous human tongue DOK cells. We also established that IL-6 is driving *anoikis* resistance in an autocrine fashion and that anti-IL-6 neutralising antibodies, anti-IL-6 receptor antibodies and anti-TLR2 receptor antibodies inhibit *anoikis* resistance in cancerous SCC-4 human tongue cells. Mitochondrial oxygen consumption (and NADH-related metabolism) was lower and extracellular acidification flux higher in cancerous cells. Oxygen consumption in SCC4 cells was not influenced by endogenously produced Il-6 but was inhibited by added recombinant human (rh) IL-6.
Conclusions: The data suggest a promising role for anti-IL-6 receptor antibody and anti-TLR2 receptor antibody in treatment of oral cancer.

56ASM-0233 S2 IS | Mitochondrial Her2 promotes tumorigenicity by stimulating respiration

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Amplification of a receptor tyrosine kinase ERBB2/HER2 is associated with aggressive disease and resistance to therapy in breast cancer. HER2 is localized mostly at the cell membrane, but a fraction of the HER2 protein also translocates into mitochondria. Nevertheless, if and how mitochondrial HER2 contributes to tumorigenicity remains unknown. To address these questions, we enriched the mitochondrial fraction of HER2 using the N-terminal targeting sequence of COX8 in breast cancer cell lines. We document that mitochondrial (mt)HER2 stimulates bioenergetics by promoting supercomplex assembly and enhances proliferation/invasiveness in vitro and tumor growth/metastatic potential in vivo, in a kinase activity-dependent manner. On the other hand, constitutively active mtHER2 provokes excessive mitochondria ROS generation and sensitizes to cell death, suggesting that HER2 in mitochondria needs to be tightly controlled. Mechanistically, mtHER2 seems to associate with and phosphorylates NDUFA13 (Grim19), a subunit of respiratory complex I, contributing to CI assembly. Accordingly, mtHER2 promotes tumorigenicity by supporting bioenergetics and optimal redox balance.

56ASM-0178 S2 IS | Mitochondrial dysfunction and energy crisis in cancer cachexia

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The occurrence of cachexia significantly complicates patient management, also impacting on anticancer treatment tolerance and response, eventually resulting in reduced quality of life and survival. Cachexia is associated with loss of body weight and muscle mass. This latter mainly results from protein hypercatabolism, however the underlying mechanisms are not fully elucidated and targeted therapies are still lacking. Impaired muscle mitochondrial function has been proposed to play a role in cancer-induced muscle wasting, markedly contributing to fatigue and asthenia in cancer patients. Quite recently, tumor-derived extracellular vesicles (TMVs) have been shown to retain the potential to modulate energy metabolism in myocyte cultures and to recapitulate some of the typical features of cachexia when infused into healthy animals. The mechanisms underlying such effects are still not clear; however, TMVs contain several small non-coding (snc) RNAs that could actively contribute to generate the observed phenotype.

Several strategies can be considered to boost mitochondrial function. In this regard, PGC-1α overexpression improves mitochondrial biogenesis and overall mitochondrial mass, but is not able to prevent the loss of muscle mass and strength in tumor-bearing mice. Conversely mild exercise training, combining endurance and resistance components, improves muscle wasting and the loss of muscle strength, sustaining mitochondrial function. However, frail cancer patients could be unable to cope with exercise. For this reason, the effectiveness of exercise-mimicking drugs in improving cachexia is currently being investigated .

On the whole, the optimization of integrated multimodal anti-cancer and anti-cachexia approaches, including tools able to modulate TMV secretion and/or fusion with myofibers or to antagonize specific sncRNAs vehicle by TMVs, will likely provide new bases to improve the management of cancer patients.

56ASM-0144 | Oxygen differentially affects gene expression, cell bioenergetics, and mitochondrial networks in distinct cancer cell lines

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Background: Although tissue oxygen levels range from 1-6% (physioxia), cell culture is routinely performed at supraphysiological concentrations (~18%), leading to altered oxygen-consuming processes such as reactive oxygen species (ROS) production. We thus aimed to investigate how oxygen affects energy metabolism, mitochondrial networks, and gene expression, and whether such responses are similar across cell lines, or cell-line specific.

Materials and Methods: We cultured LNCaP, Huh-7, PC-3, and SH-SY5Y at either 5% or 18% oxygen for two weeks. Through RNAseq and bioinformatics tools, we performed differential gene expression and functional enrichment analyses. Effect of oxygen levels on bioenergetics and mitochondrial networks was studied in LNCaP and Huh-7 cells. Cellular respiration was measured by Seahorse extracellular flux analysis, whereas mitochondrial network dynamics was assessed using fluorescence microscopy and mitochondrial network analysis (MiNA).

Results: We found oxygen affected transcript abundance of hundreds of genes, of which 88.5% were cell-type specific,

evidencing little overlap among cell lines. Functional enrichment analysis revealed Huh-7 to be the most sensitive to oxygen, showing altered translation, ROS formation, mitochondrial respiration, drug metabolism, and extracellular matrix organization. PC-3 cells also showed altered respiration and ROS formation. Oxygen concentration was found to affect the TGF-β pathway in LNCaP cells and neurogenesis in SH-SY5Y. Finally, we observed differences in bioenergetics and mitochondrial network characteristics of LNCaP and Huh-7 cells.
Conclusions: We conclude that cellular responses to oxygen in the 5-18% range are extensive and cell-type specific, highlighting the importance of maintaining physioxia in cell culture.

56ASM-0274 | The role of aspartate synthesis in tumor development

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Background: Proliferative cells such as cancer cells require both energy production and biosynthesis in order to proliferate and form tumors. Mitochondrial oxidative phosphorylation (OXPHOS) system is important for both these function, and in the complete absence of OXPHOS cancer cells cannot form tumors in mice. Previous research demonstrated that pyrimidine synthesis (PS) driven by OXPHOS is the essential consequence of OXPHOS function and is required for tumorigenesis. Besides directly driving the PS enzyme Dhodh, OXPHOS is also essential for PS because NADH/NAD+ recycling at complex I allows production of aspartate. However, it is unclear if complex I deficiency is equivalent to the deficiency of aspartate biosynthesis with respect to in vivo tumor growth.
Materials and Methods: B16 murine melanoma cell line was used to generate knock-out models using CRISPR-Cpf1 system. Tumor were generated by subcutaneous injection of B16 cell line in C57BL/6 mice. Intracellular levels of metabolites were detected using GC-MS.
Results: The tumor growth results revealed that aspartate deficiency does not fully recapitulate complex I defects, an important issue not only for basic understanding of the OXPHS system, but also for effective development of anticancer agents directed at complex I and PS. Lower levels of aspartate detected in metabolic analysis, from both *in vivo* tumor samples and *in vitro* cell culture, show that aspartate is not crucial for proliferation and tumor formation, and that in case of Got2-1 dKO tumor proliferation is rescued trough upregulation of other metabolic pathways.
Conclusions: Alternative pathways that bypass aspartate synthesis deficiency and provide pool of metabolic intermediates, that are required as substrate for building blocks are yet to be defined and described in our model. This presents an important insight in mechanism how tumors can bypass metabolic dependencies but also to reveal metabolic vulnerabilities that can be used as potential targets for anticancer treatment.

56ASM-0289 S2 IS | Metabolic switch utilizing ammonia supports proliferation in regenerating liver

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Our research has documented horizontal transfer of mitochondria in a mouse model of cancer from stroma to cancer cells with compromised oxidative phosphorylation (OXPHOS) in order to restore respiration. Functionally, the restoration of the ability to respire was attributed to the OXPHOS-linked activity of dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme catalysing the 4th reaction of the *de novo* pyrimidine synthesis. One biological event epitomized by high proliferation is liver regeneration after partial hepatectomy (PHx), with liver re-growth after 60% PHx in mouse in 5-6 days. As this process requires robust formation of nucleotides, we found that blocking the *de novo* pyrimidine synthesis prevents the regeneration process. Additionally, to support the massive requirement for proliferation, the liver switches from ammonia detoxification to diversion of ammonia into metabolic pathways converting it to substrates such as glutamate and glutamine, for amino acids and nucleotide syntheses. Thus, to sustain the high level of proliferation in regenerating liver, metabolism switches to anabolic pathways, also making use of ammonia that would otherwise be converted into a waste product.

56ASM-0244 S2 IS | Gut microbiome-midbrain mitochondria axis in gut-first PD

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Recent evidence suggests that gut microbiota regulates intestinal homeostasis by the modulation of immune responses. A gut-derived inflammation may impact the brain with vast implications in neurodegenerative disorders. It has been reported, at least for some Parkinson's disease (PD) patients that intestinal dysbiosis arises years before clinical diagnosis, which lead us to propose that PD patients gut microbiota-mediated intestinal immune alterations triggers PD-related neurodegeneration. To test this hypothesis, we colonized wild-type mice with fecal material from a healthy-control and a PD patient to challenge the gut-immune-brain axis. PD gut microbiome induced the loss of TH+ cells and motor dysfunction. Indeed, we observed a significant decrease of Segmented Filamentous Bacteria in the ileum-associated mucosa of PD transplanted mice, which correlates with an increase in gut inflammation, intestinal barrier disruption, CD4+ infiltration and a decrease in Th17 homeostatic cells in the ileum. In this regard, we found a decrease in CD4+ cells and an increase in pro-inflammatory cytokines in the blood of PD transplanted mice that could contribute to an increase in the permeabilization of the blood-brain-barrier, observed by an increase in mesencephalic Ig-G-positive microvascular leaks and by an increase of mesencephalic IL-17 levels. Indeed, we observed a decrease in mitochondrial function that in the midbrain may be involved in the activation of immune and inflammatory responses. Remarkably, this a caudo-rostral mitochondrial dysfunction correlated with an accumulation of mice aSynuclein aggregates. Our findings reveal that PD gut microbiota induces the pathogenesis of PD in wild-type mice, providing new insights into the relationship between the gut-immune-brain axis.

56ASM-0255 S2 IS | PNC2 (SLC25A36) deficiency associated with the hyperinsulinism/hyperammonemia syndrome

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The hyperinsulinism/hyperammonemia (HI/HA) syndrome is the second most common form of congenital hyperinsulinism. Children affected by this syndrome have both fasting and protein sensitive hypoglycemia combined with persistently elevated ammonia levels. Mutations in *GLUD*1, coding for the mitochondrial enzyme glutamate dehydrogenase, cause the HI/HA syndrome. These are dominantly expressed, missense variants that increase enzyme activity by reducing its sensitivity to allosteric inhibition by guanosine triphosphate (GTP).

We have investigated two siblings who presented with the biochemical features of HI/HA syndrome but did not carry pathogenic variants in *GLUD*1. By whole exome sequencing a homozygous splice site variant was identified in solute carrier family 25, member 36 (*SLC*25*A*36). *SLC*25*A*36 encodes the pyrimidine nucleotide carrier 2 (PNC2), a mitochondrial nucleotide carrier that transports pyrimidine as well as guanine nucleotides across the inner mitochondrial membrane. Allelic segregation confirmed autosomal recessive transmission of this variant which leads to a 26 aa in-frame deletion in the first repeat domain of the protein. Yeast complementation studies and direct transport assays on the recombinant protein demonstrated its deleterious effect on transport activity. Furthermore, knockdown of *slc*25*a*36 expression in HeLa cells caused a dramatic reduction in the mitochondrial GTP content which likely leads to an hyperactivation of GDH (Shahrour et al. J Clin Endocrinol Metab. 2021 Dec 31:dgab932).

Over the course of these studies, a second homozygous mutation c.803dupT, p.Ser269llefs*35 in the SLC25A36 gene has been found in an unrelated patient (Jasper et al. Int J Mol Sci. 2021;22(18):9929) displaying the features of HI/HA syndrome and, in addition, hypothyroidism, chronical obstipation, along with language and general developmental delay. Supplementation with oral uridine led to an improvement of the latter symptoms (but not HI/HA).

These findings underscore the importance of nucleotide metabolism in the pathogenesis of HI/HA and expand the spectrum of inborn errors of mitochondrial transporters.

56ASM-0025 | Defective mitophagic clearance of damaged mitochondria promotes extracellular vesicle-mediated release of mitochondrial components in huntington's disease

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Background: Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion at the HTT gene that leads to an abnormally long polyglutamine expansion in the huntingtin protein. Accumulation of dysfunctional mitochondria in HD can result from both an accumulation of mitochondria damage and compromised mitophagy.

Materials and Methods: In this work, we analyzed the mitochondrial function/dynamics of differentiated HD neuronal-like cells and further assessed auto(mito)phagy in fibroblasts of premanifest and manifest HD patients and performed a proteomic and genomic profile of small extracellular vesicles (EVs). We investigated the presence of mitochondrial components in human EVs and the relation to the dysfunction of both mitochondria and autophagy pathways.

Results: We observed that HD cells exhibited impaired mitochondrial function/dynamic and auto(mito)phagy associated with increased EV secretion. EVs cargo isolated from HD-fibroblasts and neuronal-like cells was enriched in mitochondrial proteins and mtDNA. Further genomic analysis demonstrated the presence of mtDNA also in neuron-derived EVs (NDE) isolated from the patients' plasma. Notably, mtDNA copy number was increased in HD-EVs supporting this pathway as a possible complementary route to discard mitochondrial material with reactive potential in HD pathophysiology.

Conclusions: This study provides a novel framework connecting EVs enhanced release of mitochondrial components to mitochondrial and lysosomal dysfunction in HD.

56ASM-0186 | Mitochondria-associated membranes (MAMs) and sterile inflammation in bipolar disorder

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Background: Bipolar disorder (BD) is a neuropsychiatric illness associated with cognitive and functional impairments. Recent evidences support that BD-related brain structural alterations and cognitive deficits involve impairments in mitochondrial function, stress responses and inflammation. Innate immunity is regulated by endoplasmic reticulum (ER)-mitochondria contact sites, the Mitochondria-Associated Membranes (MAMs), and miscommunication between both organelles have been implicated in the pathophysiology of several diseases. This study aimed to investigate the link between MAMs and inflammation in BD.

Materials and Methods: Fibroblasts from BD patients and healthy controls were used as *in vitro* models. ER-mitochondria tethering was assessed by confocal microscopy and transmission electron microscopy (TEM). Concomitantly, inflammatory profile was investigated by measuring mRNA levels of inflammatory cytokines (e.g. TNFa, IL-8, IL-6). Pro-IL-1b and NLRP3 mRNA levels, and secreted IL-1B were quantified as a measured of NLRP3 inflammasome activation.

Results: BD patients-derived fibroblasts revealed increased ER-mitochondria coupling, which was further strengthened by TEM showing increased MAMs number. Regarding inflammation, it was observed an increase in basal levels of pro-inflammatory cytokines in BD cells. However, NLRP3 inflammasome activation, which is a stress response, was found attenuated in BD as supported by downregulation of secreted IL-1b. This finding was corroborated by data obtained in innate immune cells, namely monocytes derived from BD patients *versus*

healthy controls, suggesting impaired activation of NLRP3 inflammasome in BD.

Conclusions: This study provides evidence that impaired communication between ER and mitochondria at MAMs is implicated in BD pathophysiology and is associated with deleterious processes including a pro-inflammatory status.

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56ASM-0093 S2 IS | Measuring cell energy production to assess whole- and sub-cellular metabolism

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Better assays of cellular energy metabolism pathways are needed to detect altered metabolism that underlies many important human disorders including cancers, immune and neurological deficiencies, diabetes, obesity, and others. To complicate metabolic energy profiling, the main energy metabolism pathways in eucaryotic cells reside in two different cellular compartments separated by different membranes and governed by different transporters. Glycolysis pathways are in the cytoplasm and the TCA Cycle and Electron Transport Chain are in the mitochondria. Metabolomic pool measurements have fundamental limitations in measuring energy pathways. They are performed with cell extracts in which all membranes are solubilized, thereby destroying the compartments and combining all metabolite pools. We have developed a simple and gentle alternative assay technology that preserves compartmentation and enables measurement of the various energy pathways, simultaneously measuring rates of metabolism of extracellular substrates feeding the cytoplasm and intracellular substrates feeding the mitochondria. Using this technology one can scan cells to determine which pathways are operative and how the pathway activities change due to genetic alterations, epigenetic gene regulation, and exposure to drugs or hormones. This presentation will review the technology and show examples of how it can inform basic and advanced cellular studies.

56ASM-0083 | Untangling the protective role of Zfra 1-31 peptide against type 2 diabetes-associated brain damage: preliminary results of a pilot study

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Background: Type 2 diabetes (T2D) is a major risk factor for cognitive decline and dementia, including Alzheimer's disease (AD). So, it becomes urgent to clarify the mechanisms underlying T2D-associated brain damage to find effective strategies to avoid/delay this complication. Recent evidence shows that WW domain-containing oxidoreductase1 (WWOX) overexpression/overactivation plays a pivotal role in the development of insulin resistance, a common feature between T2D and AD. Therefore, we hypothesize that Zfra1-31 peptide, a specific inhibitor of WWOX, can be a potential therapeutic strategy against T2D-associated brain damage and cognitive decline.

Materials and Methods: C57BL/6J mice were divided in 4 groups: 1) normal control diet (NCD); 2) NCD treated with 2mM Zfra1-31 (4x; 1 injection/week via tail vein); 3) HFD (58kcal% Fat/Sucrose) for 16weeks; 4) HFD treated with Zfra1-31. Blood glucose dyshomeostasis was assessed by glucose tolerance tests and measurement of insulin and glycated hemoglobin levels by ELISA and enzymatic methods. Then, we will perform a battery of behavioral/cognitive tests and will evaluate mitochondrial function using a XF24 Extracellular Flux Analyzer and key proteins involved in WWOX signaling, synaptic integrity and learning/memory by western blotting.

Results: We observed that HFD induced a T2D phenotype as shown by the increase in mice body weight, peripheral blood glucose and glycated hemoglobin levels and decreased glucose tolerance. Soon, we expect to demonstrate that HFD-induced T2D involves WWOX activation in brain tissue contributing to mitochondrial dysfunction, cognitive/behavioral defects and loss of synaptic integrity being these alterations counteracted by Zfra1-31.

Conclusions: We showed that HFD induces a phenotype of T2D and, shortly, we expect to demonstrate that T2D-associated brain defects are significantly ameliorated by Zfra1-31.

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56ASM-0198 | Metabolic fingerprint during early stages of osteoclastogenesis and the modulator role of estradiol: new insights in bone metabolism

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Background: Estrogens maintain bone mass and protect the adult skeleton by suppressing the bone-resorbing cells, the osteoclasts. RANKL, the cytokine responsible for osteoclast differentiation, increases mitochondria mass and activity. These stimulatory effects were found as early as 48 h. 17β-estradiol (E2) signaling in osteoclast precursors prevents these effects and activates the mitochondrial apoptosis pathway. Herein, we aimed to elucidate the early effects of RANKL and E2 on osteoclast progenitor metabolism, mitochondria, and apoptosis, using RAW 264.7 macrophage cell line and bone marrow-derived macrophages.
Materials and Methods: Bone marrow-derived macrophages and RAW 264.7 cell line were cultured with RANKL (30 ng/ml) and estradiol (E2) (10^{-8}M). Metabolic profile and ATP production were assessed by measuring oxygen consumption and extracellular acidification rates using the Seahorse XFe96 Extracellular Flux Analyzer. Metabolic pathways were measured using S-1 MitoPlates. ATP levels were measured by CellTiter-Gloâ Luminescent Cell Viability Assay. NADH oxidation to NAD^+ was used to evaluate Complex I activity. Total and mitochondrial ROS and mitochondrial membrane potential were measured with H_2DCFDA, MitoSOX, and TMRM probes, respectively. Cell number was evaluated by Hoechst staining. Cytochrome C, Bax, and Bak expression were evaluated by western blotting. The cleavage of DEVD-AFC was used to measure Caspase-9 and Caspase-3 activity.
Results: RANKL stimulated mitochondrial respiration, complex I activity, and ATP production as early as 3 h. RANKL-stimulated cells showed an increased capacity to oxidize TCA cycle substrates, fatty acids, and amino-acids. E2 suppressed the stimulatory actions of RANKL. E2 in the presence of RANKL also decreased cell number and stimulated the mitochondrial-mediated apoptotic pathway, detected as early as 3h. The pro-apoptotic effects of E2 were associated with an increase in cytosolic and mitochondrial calcium levels and accumulation of p392S-p53 in mitochondria.
Conclusions: These findings elucidate the early effects of RANKL on osteoclast progenitor metabolism and suggest novel mechanisms that contribute to postmenopausal osteoporosis

56ASM-0219 | Detraining of the heart: impact of exercise practice exclusively during gestational diabetes mellitus on maternal cardiac mitochondria

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Background: Gestational diabetes mellitus (GDM) is the most common metabolic pregnancy disorder. GDM-mothers have an increased risk of developing cardiovascular diseases (CVD) after pregnancy. Mitochondrial dysfunction is implicated in CVD. Exercise is recommended as a non-pharmacological therapy to improve GDM-metabolic homeostasis. However, the implications of exercise practice exclusively during pregnancy on cardiac function were not yet disclosed. We identify here cardiac mitochondrial effects of exercise practice during GDM-pregnancy that remained for 8-weeks after cessation.
Materials and Methods: Sprague-Dawley females were fed a control(C) or high-fat-high-sugar diet(HFHS) to induce GDM and subjected to sedentary behavior(S) or exercise(E). Exercise protocols ceased upon delivery and females euthanized at 25-weeks (8-weeks postpartum). Cardiac and plasma tissue were collected. Glucose tolerance was evaluated before and at mid-pregnancy.

Gestational weight gain(GWG), litter size, cardiac mitochondrial oxygen consumption rates, and membrane potential were assessed. The levels of plasma protein expression and mRNA transcripts were evaluated. Data were analyzed using Mann-Whitney or t-student tests(n≥6) according to Gaussian data distribution, p≤0.05 considered statistically significant.

Results: At mid-pregnancy, HFHS-groups showed impaired glucose metabolization (HFHS-S, $p = 0.01$, HFHS-E, $p = 0.02$). Exercise prevented GDM-induced excessive GWG($p = 0.004$), but not the increase in litter size(vs.C-S, $p = 0.03$). Increased GDM-S plasma glucose and triglycerides($p = 0.005$, $p = 0.002$), were prevented by exercise, contributing to decreased levels of inflammatory markers (vs.GDM-S, IL-6: $p = 0.032$, TNF-α: $p = 0.015$). Hearts from the GDM groups showed increased phosphorylated/total-Akt(Ser473) ratio (GDM-S, $p = 0.027$, GDM-E, $p = 0.03$). PGC-1α transcripts were increased in GDM-E($p = 0.006$). Cardiac mitochondria from GDM-mothers phosphorylated ADP slower (vs.C-S,$p = 0.01$), aggravated by exercise (vs.C-S,$p = 0.003$), and accompanied by decreased RCR (vs.C-S, $p = 0.03$) when using complex-I substrates.

Conclusions: Cardiac mitochondria from GDM-mothers remained affected by exercise practice during pregnancy 8-weeks postpartum. Despite improvements in physiologic and plasma parameters, cardiac mitochondria revealed decreased efficiency. This could derive from exercise-induced cardiac remodeling regression due to exercise abstinence, or a limited ability of GDM-exercised hearts to cope with simultaneous challenges, i.e. pregnancy. Recommendations of exercise practice in GDM management should be carefully evaluated.

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56ASM-0232 S2 IS | Mitochondriotropic Antioxidants Based on Hydroxycinnamic Acids Activates Nrf2-Mediated Cell Signalling Responses to Oxidative Stress: Implications in Non-Alcoholic Fatty Liver Disease (NAFLD)

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Background: Mitochondria-targeted hydroxycinnamic acid derivative ($AntiOxCIN_4$) can alter the cellular redox and energetic status and activate mitochondria-to-nucleus signaling pathways, such as Nrf2/Keap1 pathway. Understanding such mechanisms is crucial to potentiate the therapeutic application of mitochondriotropic antioxidants towards specific pathological conditions. 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. Here, we investigated the potential effect of $AntiOxCIN_4$ in the prevention of NAFLD development.

Materials and Methods: Human hepatoma-derived cell line HepG2 was treated with $AntiOxCIN_4$ for 48h in the presence and absence of fatty acid (FA) overload. Additionally, C57BL/6J mice daily supplemented with 2.5 mg $AntiOxCIN_4$ were then fed with standard diet (SD) or Western diet (WD) (30% high-fat, 30% high-sucrose) for 16 weeks.

Results: After an initial decrease in oxygen consumption paralleled by a moderate increase in superoxide anion levels, AntiOxCIN4 led to a time-dependent Nrf2 translocation to the nucleus. AntiOxCIN4 treatment enhanced mitochondrial quality by triggering the clearance of defective organelles by autophagy and/or mitophagy, coupled with increased mitochondrial biogenesis. AntiOxCIN4 have the ability to maintain hepatocyte redox homeostasis, up-regulate cellular antioxidant defense system, regulate the electrophilic/nucleophilic tone, and protected HepG2

cells against the detrimental effects of FA overload. In a WD-fed mice model, AntiOxCIN$_4$ decreased body weight gain and hepatic steatosis by decreasing LD number/size and its composition. These effects were correlated with increased cellular FAO activity.

Conclusions: Although the cellular mechanisms behind NAFLD pathogenesis are still a focus of controversy, the multi-operational mechanism of action of AntiOxCIN4, make this molecule a valuable drug candidate in the context of metabolic-related hepatic disorders, such as NASH/NAFLD.

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56ASM-0177 S2 IS | The DecylTPP mitochondria-targeting moiety lowers electron transport chain supercomplex levels in primary human skin fibroblasts

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Attachment of cargo molecules to lipophilic triphenylphosphonium (TPP^+) cations is a widely applied key technology for mitochondrial targeting. We previously demonstrated that the vitamin E-derived antioxidant (Trolox) increases the levels of active mitochondrial complex I (CI), the first complex of the electron transport chain (ETC), in primary human skin fibroblasts (PHSFs) of Leigh Syndrome (LS) patients with isolated CI deficiency. Primed by this finding, we here studied the cellular effects of mitochondria-targeted Trolox (MitoE10), mitochondria-targeted ubiquinone (MitoQ10) and their mitochondria-targeting moiety decylTPP (C_{10}-TPP^+). Chronic treatment (96 h) with these molecules of PHSFs from a healthy subject and an LS patient did not greatly affect cell viability. Unexpectedly, this treatment reduced CI levels/activity, lowered the amount of ETC supercomplexes, inhibited mitochondrial oxygen consumption, increased extracellular acidification, altered mitochondrial morphology and stimulated the levels of hydroethidine-oxidizing ROS. We conclude that the mitochondria-targeting decylTPP moiety is responsible for the observed effects and advocate that every study employing alkylTPP-mediated mitochondrial targeting should routinely include control experiments with the corresponding alkylTPP moiety.

56ASM-0140 S2 IS | Targeting mitochondria in neurodevelopmental diseases. a focus on down, rett and fragile X syndromes

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Mitochondria play a pivotal role in cellular energy-generating processes acting as central hub integrating signaling networks in several metabolic pathways controlling neurogenesis and neuroplasticity; consequently, disturbance in mitochondrial function and signaling leads to change in neural cell fate and negatively affects neurogenesis and neuroplasticity processes (1). In this contest, we have studied whether and how deficit in mitochondrial function is critical in the pathogenesis of some neurodevelopmental diseases as Down syndrome (DS), Rett syndrome (RTT) and Fragile X syndrome (FRAX), with the aim to improve some neurological processes by targeting mitochondrial bioenergetics.

We have identified and characterized the molecular mechanisms responsible for mitochondrial dysfunction and energy deficit occurring in whole brain, in specific brain areas or in neural progenitor cells of mice modelling DS (Ts65Dn mice) (2, 3), RTT (MeCP2-deleted mice) (4) and FRAX (*Fmr*1 null mice) (5), as well in human fibroblasts (6) and lymphocytes from DS children (7).

As common features shared in these genetic syndromes of different aetiology, a deregulation of selective signaling pathways controlling mitochondrial function (including cAMP/PKA, PGC1a/SIRT1/AMPK signaling pathways) occurred, leading to an impairment of mitochondrial oxidative phosphorylation apparatus. This resulted in cell and brain energy deficit beside to radical species overproduction and oxidative stress. We disclosed the capability of some bioactive drugs of natural origin, such as some polyphenols in DS (8, 3) or drugs already used for treatment of other diseases, such as metformin in RTT (9), to target selective mitochondrial regulatory signaling pathways, to reverse mitochondrial impairment and to improve neurobiological alterations and some neurobehavioral phenotypes.

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56ASM-0187 | Targeting neuroinflammation and modulating iron dysregulation with innovative multitarget therapeutics for amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a fast progressing and devastating disease characterised by the progressive loss of motor neurons in the brain stem, spinal cord and motor cortex. The patients rapidly lose their voluntary movements and die due to respiratory failure. The drugs available for symptomatic treatment of the disease, Riluzole and Edaravone, only provide modest benefits to the patients.

The NF-kB transcription factor is a critical regulator of inflammation in ALS and its inhibition showed benefits in animal models of ALS. Additionally, abnormal iron accumulation has been associated with ALS pathology. Hence, following these evidences, we propose the development of multitarget small-molecules capable to modulate NF-κB activity and, at the same time, presenting the neuroprotective properties of iron chelators as a novel therapeutic solution for ALS.

Materials and Methods: We started by designing a library > 200 multitarget compounds with iron chelator groups and that could interact with IKKB, a protein crucial for NF-kB canonical pathway. The initial virtual screening studies led us to prioritise a set of 20 compounds for which we evaluated their iron chelation and antioxidant properties using cell-free assays. The cytotoxicity and capacity of the most promising compounds to protect neuronal cells from oxidative stress and ferroptotic induced death was evaluated *in vitro*. The ADME profile of selected candidates was evaluated using biomimetic approaches.

Results: Following a multidisciplinary approach, we synthesised a series of novel multitarget compounds and identified in our preliminary in vitro screening we identified three promising compounds (**8**, **12** and **14**). The identified candidates presented higher passive permeability through physiological membranes, including BBB, and protected neuronal cells from oxidative stress damage better than Edaravone and Deferiprone (iron chelator in clinical trials for ALS).

Conclusions: The initial results of our project suggest that our approach may be further explored and lead to an innovative therapeutic approach for ALS patients.

56ASM-0223 | Impaired bioenergetic profile in neuron progenitor cells from iPSC's of patients affected by AGC1 deficiency

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Background: AGC1 deficiency is a rare encephalopathy (DEE39, OMIM# 612949) that manifests in infants with hypomyelination associated with reduction of N-acetyl-aspartate, the precursor of the myelin lipids. AGC1 deficiency is caused by mutations of *SLC25A12* gene, encoding the isoform 1 of the mitochondrial aspartate/glutamate carrier (AGC1). AGC1 catalyzes the entry of glutamate into mitochondria in exchange for aspartate and is essential for the oxidation of glucose in neurons and the import of the glycolysis-derived reducing equivalents in the mitochondrial matrix since it is a component of the malate-aspartate NADH shuttle.

Materials and Methods: We reprogrammed fibroblasts of two patients carrying different missense mutations in *SLC25A12* into iPSCs for the subsequent differentiation in neuron progenitor cells (NPCs).In NPCs of a patient expressing the R353QAGC1 mutant with a residual transport activity and NPCs of a new identified patient carrying compound heterozygote mutations (c.225del; p.(Glu76Serfs*17) and c.1747C > A; p. (=)) that totally impede the expression of the carrier, proliferative and bioenergetic parameters were measured.

Results: Both patient NPCs revealed a proliferation deficit, when deprived of glutamine, with higher cell death, as compared to control NPCs. Along with higher lactate production and increased glycolytic activity and total ATP production, both patient NPCs showed a dramatic reduction of mitochondrial respiration, as measured in the presence of glucose alone or in combination with other respiratory substrates. Since the administration of ketogenic diet appears to improve myelination in the patients with AGC1 deficiency, we evaluated the effect of ketone bodies on NPCs mitochondrial respiration. In patient NPCs, ketone bodies significantly enhanced mitochondrial respiration, but only in the absence of glucose and in particular in combination with glutamine.

Conclusions: Overall, our data suggest that NPCs of patients benefit from a metabolism where the mitochondrial oxidation of pyruvate and NADH is limited and alternative precursors of mitochondrial acetyl-CoA and of TCA cycle intermediates are provided.

56ASM-0043 | Mitochondrial uncoupling proteins (UCPs) are metabolic modulators in human sertoli cells

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Background: Mitochondrial Uncoupling Proteins (UCPs) are proton channels present in the mitochondrial inner membrane. Currently, six homologues (UCP1-6) have been identified. UCPs are regulators of oxidative phosphorylation, reactive oxygen species (ROS) production, and, consequently, the cellular redox state. UCPs dysfunction is associated with the onset of metabolic diseases, leading to mitochondrial dysfunction and increased oxidative stress. Male infertility is an overlooked comorbidity of metabolic diseases, due to the sensitivity of the testis to oxidative stress and metabolic dysfunction. However, the expression and function of UCPs in the human testis are unclear.

Materials and Methods: Primary cultures of human Sertoli cells (hSCs) were established from testicular biopsies of healthy men with conserved spermatogenesis seeking fertility treatment ($n = 6$). Total RNA was extracted and UCP homologues (UCP1-6) mRNA expression was accessed by RT-PCR. UCP1-3 protein expression was accessed by immunofluorescence. Then, hSCs were treated with genipin (0.5, 5, 50, and 100 μM), a selective UCP inhibitor, for 24 h. Cellular viability and proliferation were evaluated. Total intracellular ROS production was evaluated through the CM-H2DCFDA probe. Mitochondrial membrane potential and function were analysed by JC-1 assay and Seahorse XF Cell Mito Stress assay, respectively. Culture media were collected and analysed by ^{1}H-NMR.

Results: We were able to detect the mRNA expression of all UCP homologues (UCP1-6) in hSCs. The protein expression of UCP1-3 was also identified. The inhibition of

UCPs by genipin decreased cellular proliferation although no cytotoxicity was observed. Additionally, UCPs inhibition decreased the mitochondrial membrane potential and mitochondrial function in a dose-dependent manner.
Conclusions: We were able to identify, to the best of our knowledge for the first time, the expression of the different UCP homologues (UCP1-6) in hSCs. We demonstrated that UCPs are important modulators of hSCs' mitochondrial activity and metabolism. Our results suggest that UCPs dysfunction could play a significant role in metabolic diseases-related male infertility.

56ASM-0240 | Statistical analysis of instrumental reproducibility in high-resolution respirometry

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Background: Evaluation of instrumental reproducibility is an essential component of quality control - defined as standard operating procedures - to quantify the precision and limit of detection of an analytical procedure.
Materials and Methods: Instrumental tests implemented as standard operating procedures in high-resolution respirometry are the *sensor test* and the *chamber test*. The sensor test includes calibrations of the signal of the polarographic oxygen sensor (POS) in terms of oxygen concentration c_{O2} [μM] to evaluate the performance of the POS. The chamber test (instrumental O_2 background test) focuses on the slope dc_{O2}/dt to determine oxygen consumption by the POS and backdiffusion into the chamber [1]. We evaluated instrumental tests of 48 Oroboros O2k chambers obtained from a 3-year study on MiR05-Kit (Oroboros Instruments), carried out in the absence of sample.
Results: Stability of oxygen calibration signals at air saturation and zero oxygen was monitored up to 8 months. The maximum drift over 1 to 3 days was 0.05 $pmol \cdot s^{-1} \cdot mL^{-1}$, with no persistence over time since drift was < 0.004 $pmol \cdot s^{-1} \cdot mL^{-1}$ for a time interval of one month, corresponding to a drift per day of 0.2 % of the signal at air saturation. Instrumental O_2 background dc_{O2}/dt was stable within ± 1 $pmol \cdot s^{-1} \cdot mL^{-1}$ at different O_2 concentrations when measured at monthly intervals.
Conclusions: Taken together, these results confirm the instrumental limit of detection of volume-specific O_2 flux at ± 1 $pmol \cdot s^{-1} \cdot mL^{-1}$. Following the standard operating procedures applied in the present study provides an instrumental proficiency test to ensure the unique reproducibility in high-resolution respirometry.

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56ASM-0242 | In vitro cardioprotective potential of Côa Valley (Portugal) plants extracts against lipotoxic insults

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Background: The Côa Valley region (Portugal) is a UNESCO World Heritage Site. This region has a typical dry Mediterranean climate, and the flora faces severe environmental stresses, consequently leading to the production of phytochemicals with medicinal properties. This study aims to assess *in vitro* cardioprotective activity of extracts from some regional plant species against *in vitro* lipotoxicity.
Materials and Methods: H9c2 cardiomyoblasts were grown in low-glucose Dulbecco's Modified Eagle's Medium and supplemented with 10%FBS. The tested extracts were obtained from *Rumex scutatus* subsp. *induratus* (Boiss. & Reut) Nyman (infusion–RI-Inf, decoction–RI-Dec and 80% ethanol–RI-Et80) and from *Lupinus angustifolius* Blanco (infusion–LA-Inf), *Lavandula pedunculata* (Mill.) Cav. (infusion-LP-Inf) and *Cistus salviifolius* L. - infusion–CS-Inf). Palmitic acid (PA, 0.05/0.1 mM) and free fatty acids (FFA, 0.25/0.5 mM) were used to induce lipotoxicity. Cells (5000/well) were incubated with extracts for 24 h before PA and FFA treatment. The assays were carried out 24 hours after lipotoxic insult. Resazurin and sulforhodamine B assays were used to determine metabolic activity and cell mass, respectively.
Results: We initially performed cytotoxicity studies of different extract preparations on H9c2 cells. Concentrations of extracts that did not reduce metabolic activity by more than 10% were used (RI-Inf, 0.031 mg/mL, RI-Dec, 0.25

mg/mL, RI-Et80, 0.016 mg/mL, LA-Inf, 0.5 mg/mL, LP-Inf, 0.125 mg/mL, CS-Inf, 0.031 mg/mL). Although protective effects in presence of PA were not significative, protection against cell mass loss and metabolic activity caused by FFA 0.5 mM was observed with RI-Inf, RI-Et80, LP-Inf, LA-Inf and CS-Inf.

Conclusions: Although preliminary, our data shows that LP-Inf, LA-Inf, and CS-Inf demonstrate *in vitro* potential for cardioprotective activity against lipotoxicity caused by FFA. Mitochondrial studies will be performed to evaluate protection in function of that organelle.

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56ASM-0045 | Lipid metabolism dysfunction in sperm is associated with decreased motility in asthenozoospermic men

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Background: Asthenozoospermia is a common cause of male infertility that is characterized by a reduction in sperm motility, although its molecular mechanisms are poorly understood. Lipid metabolism has a crucial role in sperm motility and morphology, as in sperm capacitation, acrosome reaction, and sperm-oocyte fusion. Nevertheless, data regarding the relevance of lipid composition of human spermatozoa is still limited. Herein, we aimed to identify lipid biomarkers related to sperm motility through the analysis of sperm samples from asthenozoospermic and normozoospermic men.

Materials and Methods: Sperm samples were collected from asthenozoospermic ($n = 17$) and normozoospermic ($n = 39$) men. Sperm parameters were assessed accordingly to the WHO guidelines. Sperm polar lipids were extracted and analysed through liquid-chromatography-mass-spectrometry. Statistical analysis was performed using Mann-Whitney and Welch's t-test, principal component analysis, and Pearson correlation coefficient.

Results: Multivariate analysis showed a distinctive lipidomic profile in sperm samples from normozoospermic and asthenozoospermic men. Phosphatidylethanolamine (PE) content was increased in normozoospermic men, whereas the levels of lysophospholipids (LPLs) were increased in asthenozoospermic samples. The levels of several lipids correlated with sperm total motility. Sperm motility was positively associated with acyl-carnitine(26:6), fatty acid(20:3);3O, PE-P(36:4), PE-O(38:6), PE-O(38:7), PE-O(40:9), and PE(38:6), while the content in lysophosphatidylcholine (LPC)18:0, LPC(18:1), LPC(20:1), LPC-O(16:1) and LPC-O(18:2), lysophosphatidylethanolamine (LPE)20:1 and LPE-O(20:2) negatively correlated with sperm motility.

Conclusions: Our results show different lipidomic signatures between asthenozoospermic and normozoospermic men. The increase in LPL content suggests impaired lipid metabolism in sperm from asthenozoospermic men that might result from an increase in oxidative stress and inflammation, two major causes of this condition. Aberrant formation of LPL in sperm plasma membrane alters the membrane integrity and the function of membrane proteins essential for the maintenance of sperm motility. Overall, our data suggest that altered lipid metabolism, probably caused by an increase in oxidative stress, might be a cause for decreased sperm motility and fertilizing ability in asthenozoospermia.

56ASM-0095 | Can hyperoside supplementation of sperm media be beneficial for the preservation of mitochondrial activity and sperm metabolism under an oxidative stress condition?

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Background: Oxidative Stress (OS) is one of the major causes of male infertility. Infertile couples often recur to assisted reproductive technology (ART) to achieve a successful pregnancy. However, preparation for ART protocols increases the exposure of gametes to OS. Media supplementation with antioxidants is a strategy to overcome this problem. Thus, we investigated the impact of the flavonol hyperoside (quercetin 3-O-galactoside) on the protection of sperm against oxidative damage.
Materials and Methods: Sperm samples of twenty normozoospermic patients were supplemented with HYP (100 and 500 μM), for 1 hour, in the presence and absence of H2O2 (300 μM). Vitamin C (VC) (600 μM) was used as a positive control. To evaluate the potential sperm oxidative damage, the following parameters were assessed: total sperm motility and vitality, mitochondrial membrane potential (MMP), and metabolite identification and quantification of the media by using Proton Nuclear Magnetic Resonance (^{1}H-NMR).
Results: Supplementation with HYP (100 and 500 μM) did not induce deleterious effects to the physiology and metabolism of the spermatozoa, after 1-hour of treatment. Under an H_2O_2-induced OS condition, HYP was able to preserve sperm motility. However, this antioxidant could not prevent the decrease of MMP promoted by H2O2. Regarding metabolism, we observed an increase in pyruvate media consumption, a decrease in lactate and alanine production and an increase in acetate media levels, under oxidative stress conditions. Interestingly, the supplementation of HYP (100 μM) led to lower levels of acetate in the media.
Conclusions: From the antioxidant conditions tested, HYP (100 μM) demonstrated the best results regarding sperm preservation. We hypothesized that supplementation of media with HYP (100 μM) was more efficient in neutralizing H_2O_2 since it reduced the pyruvate-derived overflow pathway for acetate, promoted by ROS. Notwithstanding, the effects promoted by H2O2 found in mitochondrial activity and sperm metabolism could not be reversed by HYP supplementation.

56ASM-0119 | Inhibition of mitochondrial uncoupling proteins (UCPs) impairs the motility of human spermatozoa

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Background: Mitochondrial uncoupling proteins (UCPs) have several important physiological functions throughout the body, including regulation of reactive oxygen species (ROS) production and mitochondrial membrane potential. Currently, six homologues had been identified (UCP1-6). High oxidative stress is a major cause of male infertility. However, the knowledge about the presence and function of UCPs in human spermatozoa is scarce.

The aim of this study was to assess the expression and function of the different UCPs' homologues (UCP1-6) in human spermatozoa.

Materials and Methods: Highly motile spermatozoa were secluded through density gradient centrifugation from human normozoospermic seminal samples ($n = 10$). Total RNA was extracted and UCPs mRNA expression analyzed by RT-PCR. The presence of UCP1-3 protein was evaluated by immunofluorescence. Spermatozoa were incubated (3 h at 37 °C) in Biggers-Whitten-Whittingham (BWW) media with genipin, an UCP inhibitor, at increasing concentrations (in μM: 0, 0.5, 5, and 50) and their viability and total and progressive motility were assessed. CM-H2DCFDA probe and JC-1 dye were used to measure ROS production and the mitochondrial membrane potential, respectively. Resorting to ^{1}H-NMR, spermatozoa' culture media were also analysed. To better assess the loss of motility and to determine if it was reversible, motility was registered every 15 min for a total of 105 min.

Results: The mRNA expression of all UCPs' homologues (UCP1-6) were identified in human spermatozoa. The inhibition of UCPs by genipin at 50 μM resulted in decreased mitochondrial membrane potential and permanent loss of motility. However, human spermatozoa' viability, ROS production, and metabolic profile did not differ from the control group.

Conclusions: UCPs are major regulators of human spermatozoa' motility. The inhibition of UCPs also negatively affect mitochondria' activity, which reflects its importance for motility. These results highlight the potential role of UCPs (dys)function in high oxidative stress-related male infertility.

56ASM-0172 | Phenotypical alterations in human skin fibroblasts under different metabolic conditions

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Background: *In vitro* culture conditions do not match precisely the environments that cells sense in their physiological niches and may affect cellular composition and behaviour. In this work, we studied how cellular and mitochondrial morphology and function of normal human dermal fibroblasts (NHDF) respond to different glucose concentrations in the culture medium.

Materials and Methods NHDF were cultured in DMEM containing 25 mM Glucose (HGm) and gradually adapted to 5 mM Glucose (LGm) or glucose-free galactose-containing medium (OXPHOSm). We measured cell viability markers; mitochondrial, nuclear, cytoplasm, and cell area, as well the changes in mitochondrial biogenesis. Statistic comparisons were performed using Kruskal–Wallis method and Dunn's multiple comparisons test, and differences with $p < 0.05$ were considered significant.

Results: Cells cultured in OXPHOSm showed increased metabolic activity and total cellular protein content ($p < 0.01$) in OXPHOSm, compared to HGm and LGm. Also, in OXPHOSm, we observed an increase in protein content of PGC1α ($p < 0.05$) as well as in VDAC ($p < 0.05$) when compared to HGm. Cells cultured in LGm also showed an increase in VDAC protein content (($p < 0.05$) compared to HGm). In addition to an increase ($p < 0.01$) in mitochondrial biogenesis when cells are cultured in the absence of reduced glucose, the whole cell area, cytoplasm, and nucleus were increased in OXPHOS against to HGm and LGm. The mitochondrial area was also increased ($p < 0.001$) in OXPHOSm cells, when compared to HGm and LGm.

Conclusions: Culturing human skin fibroblasts in different metabolic conditions impacts cellular morphology, composition, and behaviour, including mitochondrial metabolism and activity. In the absence of glucose, the increase in mitochondrial network was accompanied by an increase in metabolic activity and overall cell size. These results reinforce the interplay between mitochondrial and cell area with energy metabolism.

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56ASM-0183 | Mitochondriotropic antioxidant AntiOxCIN4 improves mitochondrial function and prevents non-alcoholic fatty liver (NAFL)-associated autophagic blockage in western diet (WD)-fed mice

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Background: NAFLD is a worldwide public health concern, affecting approximately one-quarter of the adult population worldwide, causing a significant morbidity burden with widespread social and economic implications. NAFLD is a multifactorial disease and the hepatic component of metabolic syndrome. Although mechanisms underlying disease pathophysiology are not fully clarified, mitochondrial dysfunction and oxidative stress (OxS) are potential key players. Driven by the lack of approved pharmacological therapies, we investigated the potential effect of a mitochondria-targeted hydroxycinnamic acid derivative (AntiOxCIN$_4$) in preventing NAFLD development.

Materials and Methods: C57BL/6J mice daily supplemented with 2.5 mg AntiOxCIN$_4$ were then fed with standard diet (SD) or Western diet (WD) (30% high-fat, 30% high-sucrose) for 16 weeks.

Results AntiOxCIN$_4$ supplementation decreased body (43%) and liver weight (39%) of WD-fed mice. AntiOxCIN$_4$ also decreased plasma hepatocyte damage markers. The improvement of hepatic-related parameters was associated with a reduction of fat accumulation (570%) and the remodeling of fatty acyl chain composition compared with the WD-fed group. We have also observed increased fatty acid oxidation-related pathways in SD and WD-fed mice supplemented with AntiOxCIN$_4$. AntiOxCIN$_4$ supplementation resulted in mitochondrial metabolism remodeling by improving mitochondrial respiration and increasing OXPHOS subunits levels in mice fed with WD+AntiOxCIN$_4$, triggered by the restoration of phospholipid profile and increased PGC-1α-SIRT3 axis. Finally, AntiOxCIN$_4$ supplementation prevented the blockage of proper recycling/elimination of damaged protein/organelles in fatty livers by increasing proteolytic activity.

Conclusions: AntiOxCIN$_4$ improves the NAFL phenotype in a mouse model, highlighting its potential use for delaying its progression.

56ASM-0193 | ECSIT is essential for RANKL-induced stimulation of mitochondria and a target for the anti-osteoclastogenic effects of estrogens

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Background: Loss of estrogens at menopause potentiates osteoporosis development. Estrogens maintain bone homeostasis by decreasing the number of osteoclasts. The binding of RANKL, a cytokine indispensable for osteoclastogenesis, to its membrane receptor RANK activates multiple signaling pathways via recruitment of the adaptor protein TRAF6. Early effects of RANKL include stimulation of mitochondria. 17β-estradiol (E2) signaling prevents this effect and induces mitochondrial apoptosis. However, the molecular mechanisms responsible for these effects remain unknown. ECSIT is a complex I-associated protein that regulates immune responses in macrophages, which also recruit TRAF6. Here, we evaluated the contribution of ECSIT to the effects of RANKL and E2 during osteoclastogenesis.

Materials and Methods: Bone marrow-derived macrophages from C57BL/6 mice were cultured with RANKL (30 ng/ml) and E2 (10^{-8} M) for 6 h. IP studies were used to evaluate protein interaction. shRNA was used to silence ECSIT. Osteoclasts were enumerated after TRAP staining. OCR was measured with Seahorse XFe96 Analyzer. ATP, lactate, and NAD were measured with commercial assay kits. NADH oxidation was used to evaluate Complex I activity. Mitochondrial ROS and mitochondrial membrane potential were measured with MitoSOX and TMRM probes. The cleavage of DEVD-AFC was used to measure Caspase-3 activity.

Results: RANKL promoted ECSIT-TRAF6 interaction and consequent translocation of ECSIT to the mitochondria. E2 abrogated these effects of RANKL. Loss of ECSIT decreased oxygen consumption, complex I activity and NAD^+/NADH redox ratio. These effects were associated

with increased mitochondrial ROS and decreased membrane potential. In the absence of ECSIT, the stimulatory actions of RANKL on mitochondrial metabolism, and osteoclast formation were prevented. Instead, RANKL stimulated apoptosis of osteoclast progenitors, mimicking the effect of E2.

Conclusions: Our results suggest that inhibition of TRAF6-ECSIT interaction in osteoclast progenitors, which abrogates ECSIT translocation to the mitochondria, promotes mitochondrial dysfunction and apoptosis in osteoclast progenitors and contributes to the bone protective effects of E2.

56ASM-0195 | E2 inhibits bone resorption by impacting the NAD/NADH redox state and decreasing the activity of the mitochondria deacetylase Sirt3

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Background: Loss of estrogens at menopause causes bone loss by increasing osteoclast number and resorptive activity. The critical cytokine for osteoclastogenesis, RANKL, stimulates mitochondrial respiration in osteoclast progenitor cultures and 17β-estradiol (E2) signaling prevents this effect and induces apoptosis. Mitochondrial dysfunction decreases the NAD^+/NADH ratio and attenuates the activity of several NAD^+-dependent enzymes such as the mitochondrial deacetylase Sirt3. Mice with deletion of Sirt3 exhibit lower bone resorption. Here, we evaluated the redox changes and consequent Sirt3 activity in response to RANKL and E2 and how the modulation of NAD^+ levels impacted osteoclastogenesis.

Materials and Methods: Bone marrow-derived macrophages from C57BL/6 mice and Sirt3 KO mice were cultured with RANKL (30 ng/ml), E2 (10^{-8} M) and nicotinamide riboside (NR; 1 mM). Sirt3 activity, ATP levels and NAD were measured with commercial assay kits. Osteoclasts were enumerated after TRAP staining. OCR rate was measured with Seahorse XFe96 Analyze. DEVD-AFC cleavage was used to measure Caspase-3 activity.

Results: The addition of RANKL for 6 h promoted an increase in NAD^+/NADH ratio. E2 inhibited this effect and stimulated caspase-3 activity. Addition of the NAD precursor NR increased NAD^+ levels and restored the NAD^+/NADH ratio, which abrogated the inhibitory effects of E2 on mitochondria. NR was also able to improve osteoclast progenitor fusion, a process associated with osteoclastic activity but did not alter the apoptotic effect of E2. RANKL increased Sirt3 activity, and this effect was prevented by E2. NR treatment also prevented the E2-induced decrease in Sirt3 activity. Osteoclast progenitors lacking Sirt3 had decreased osteoclast progenitor fusion and lower resorptive activity mimicking the effects of E2.

Conclusions: Our results indicate that RANKL stimulates Sirt3 activity by increasing the NAD^+/NADH ratio. The inhibitory actions of E2 on osteoclast resorption are mediated by a decrease in NAD^+ and Sirt3 activity, while the pro-apoptotic effect of E2 is independent of NAD.

56ASM-0205 | The mitochondria-targeted antioxidant AntiOxCIN4 is beneficial against oxidative/nitrosative stress in the amyotrophic lateral sclerosis $SOD1^{G3A}$ Mouse

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by motor neuron loss, muscle weakness, atrophy, paralysis, and death. Since mitochondrial dysfunction and oxidative stress are pivotal in ALS pathophysiology, the improvement of mitochondrial function and/or antioxidant capacity may represent promising therapeutic targets against ALS progression. We hypothesized that mitochondria-targeted antioxidant AntiOxCIN4 could attenuate ALS severity. Therefore, we analyzed the role of peripherally administered AntiOxCIN4 against oxidative/nitrosative stress in the brain and skeletal muscle of $SOD1^{G93A}$ ALS mice.

Materials and Methods: Early adult symptomatic $SOD1^{G93A}$ ALS mice were injected subcutaneously with AntiOxCIN4 (0.1 mg/Kg/day), for 2 months. We used brain cortical and skeletal muscle homogenates and colorimetry/fluorimetry based methods to evaluate the effect of

AntiOxCIN4 on oxidative/nitrosative stress markers carbonyls, nitrites and hydroperoxides. We also determined the activities of the antioxidant enzymes superoxide dismutases (SOD)-1 and -2, and of glutathione reductase.

Results: Our preliminary results indicate that AntiOxCIN4 reduced nitrites and carbonyls' levels in ALS mouse brain (by 90% and 21%) and skeletal muscle (by 91% and 28%). The mitochondria-targeted antioxidant also decreased (by 42%) the brain hydroperoxides levels, and increased the activities of SOD1 and SOD2 in ALS mice (by 696% and 112%, respectively). Brain glutathione reductase activity was upregulated by AntiOxCIN4 (by 109%) in *SOD1*^{G93A} mice.

Conclusions: In sum, our preliminary results suggest that peripherally administered AntiOxCIN4 may partially improve brain and skeletal muscle oxidative/nitrosative stress, which may contribute to a delay in the progression of ALS.

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56ASM-0207 | Adenine nucleotide translocase 2 role in the metabolic profile and matrix detachment of P19 embryonal carcinoma stem cells

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Background: Cancer stem cells (CSCs) have an altered metabolism, often showing an increased glycolytic phenotype, although mitochondrial activity is not impaired. The adenine nucleotide translocase 2 (ANT2) is responsible for the ATP uptake into the mitochondria and is known to be up-regulated in several types of cancer, being associated with cell proliferation, impaired apoptosis, and metabolic changes. Thus, we aimed to evaluate ANT2 downregulation effect on the metabolic plasticity and tumorigenesis of P19 embryonal carcinoma stem cells (P19SCs).

Materials and Methods: P19SCs transfected with scramble or ANT2-siRNA were evaluated for mitochondria remodeling, cellular metabolism, and spheroid formation in the presence or absence of etoposide (0.25μM) or 2-deoxy-D-glucose (2DG, 1mM). Western blotting, seahorse XF^{e}96 cell Mito Stress, confocal microscopy, and spheroid formation assays were used. Statistical comparisons were evaluated using Student's t-test. Differences with $p < 0.05$ were considered statistically significant.

Results: Upon ANT2 silencing in P19SCs, ANT2-expression was diminished by 90% ($p < 0.001$), which was accompanied by a decrease in hexokinase II and pyruvate dehydrogenase kinase protein levels (32% and 45%, respectively, $p < 0.05$). Basal and maximal respiration, proton-leak, and ATP production-linked respiration decreased by 23-30% in ANT2-silenced cells ($p < 0.001$), as well as the mitochondrial membrane potential ($p < 0.05$). Furthermore, ANT2-silencing, ANT2-silencing+Etoposide and ANT2-silencing+2DG, promoted a decrease on spheroid formation of 31%, 64% and 77%, respectively, to the control.

Conclusions: Our findings show that ANT2-downregulation decreases P19SCs mitochondrial activity, spheroids formation by further sensitizing cells to etoposide and 2-DG drugs. These data suggest that ANT2 targeting promotes CSCs metabolic adaptations and anoikis, such that ANT2 inhibition can be a promising target towards CSCs niche.

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56ASM-0216 | BV-2 microglial cell death induced by acute exposure to methylmercury

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Background: Methylmercury (MeHg) is a dangerous environmental contaminant with strong bioaccumulation in the food chain and neurotoxic properties. In the nervous system, MeHg may cause neurodevelopment impairment and potentially interfere with innate immune response, impairing proper control of neuroinflammation and aggravating neurodegeneration. Human populations are exposed to environmental contamination with MeHg, especially in areas with strong mining or industrial activity, building public health concerns. Taking this into consideration, the aim of this work was to clarify pathways leading to acute toxic effects of MeHg exposure in microglial cells.

Materials and Methods: BV-2 mouse microglial cells were incubated with MeHg at different concentrations (0.01; 0.1; 1 and 10μM) for 1 hour prior to Lipopolysaccharide (LPS 0.5 μg/mL) exposure for 6 or 24 hours. After cell exposure, the supernatants were harvested and IL-6 and TNF-alpha production and release, ROS production, NO release, iNOS expression, metabolic activity, Propidium Iodide (PI) uptake and caspase 3 and caspase 9 activities were assessed.

Results: MeHg 10 μM induced a reduction in the production and secretion of pro-inflammatory proteins IL-6, TNF-alpha, iNOS immunoreactivity, release of NO, and ROS formation in BV-2 cells. Furthermore, MeHg 10 μM, with and without LPS stimulation, decreased metabolic activity of BV-2 and increased the number of PI-positive cells (necrotic cell death) when compared to the respective control groups. Regarding caspase 3/9 activity, we did not measure significant activation.

Conclusions: The short-term effects of a high concentration of MeHg on BV-2 microglial cells lead to impaired production of several pro-inflammatory mediators, as well as a higher microglial cell death via necrosis, compromising their neuroinflammatory response. Clarifying the mechanisms underlying MeHg-induced neurotoxicity and neurodegeneration in brain cells is relevant to better understanding acute and long-term chronic neuroinflammatory responses due to MeHg exposure.

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56ASM-0222 | Physical exercise during obesogenic pregnancy modulates young-adult offspring's cardiac mitochondrial function in a sex-specific fashion

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Background: Obesity among women of childbearing age is rapidly increasing worldwide. Maternal obesity (MO) is associated with increased offspring's cardiovascular disease(CVD) predisposition. Cardiac mitochondrial dysfunction may contribute to offspring's CVD . Maternal physical exercise during MO (MOEx) is a putative strategy to prevent MO-induced offspring's cardiac mitochondrial dysfunction. Considering the sex-specificity of cardiometabolic risk, it is relevant to define sex-specific mechanisms that lead to cardiac metabolism modulation by MO and MOEx.

Aim: Identify the MOEx offspring's cardiac mitochondrial modulation that may persist at the young-adult stage in a sex-specific fashion.

Materials and Methods: A Sprague-Dawley MO rat model was achieved by feeding a high-fat-high-sugar diet(HFHS). Six HFHS-fed-mothers were kept sedentary(MO; $n = 6$), and six exercised(MOEx; $n = 6$), other six received control chow diet(C; $n = 6$). Offspring were kept on a standard chow diet without exercise. Male and female offspring from each group(F1-C; F1-MO; F1-MOEx) were euthanised at 32-weeks-old($n = 6$ per sex) and cardiac tissue and blood plasma collected. Different blood plasma and cardiac tissue parameters were measured and unpaired t-test or Mann-Whitney statistical tests were applied ($p \leq 0.05$).

Results: Females presented increased heart/body weight ratio in each group(female *vs*. male;F1-C, $p < 0.0001$;F1-MO;$p < 0.0001$;F1-MOEx, $p = 0.0001$). Atherogenic index was decreased for females F1-MOEx *vs*. males F1-MOEx($p = 0.0069$), and for females F1-MOEx *vs*. F1-MO($p = 0.0050$). Mitochondrial Complex-II-subunit SDHA relative expression levels were decreased for females F1-MOEx *vs*. males F1-MOEx($p = 0.006$) and increased in males F1-MOEx *vs*. F1-C($p = 0.0459$). Complex-I-supported-respiratory control ratio(RCR) was increased for F1-MOEx females and males *vs*. F1-C($p = 0.0023$; $p = 0.0059$), while Complex-II-supported-RCR was only increased for males F1-MOEx *vs*. F1-C;F1-MO ($p = 0.0039$; $p = 0.005$). MFN-1 protein levels are increased for female F1-MOEx *vs*. female F1-C($p = 0.016$), while OPA1 expression levels are increased for male F1-MOEx *vs*. male F1-C($p = 0.037$).

Conclusions: MOEx modulation of the cardiac mitochondrial bioenergetics and fusion proteins are evident in offspring's hearts in the young-adult stage and are sex-specific. These alterations may be favorable enough to improve MO offspring's cardiovascular health and decrease CVD risk.

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56ASM-0225 | Impact of extended passaging on the BEAS-2B cell line, an in vitro model of human bronchial epithelium

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Background: The BEAS-2B continuous cell line, established as an *in vitro* model of human bronchial epithelium for the study of lung carcinogenesis, is now widely used in several research contexts. Different studies employing this cell line sometimes yield conflicting results, which may be caused by the use of cultures in different passage stages.

Materials and Methods: Cultures of BEAS-2B cells at different passage stages, established and maintained in parallel, were analysed in terms of the following markers

of transformation degree: morphology and pattern of growth (light microscopy), cell size (light microscopy and Bradford method), stress response status (ELISA assay for heat shock protein 90 alpha (Hsp90α) intracellular protein levels), lactate production (enzymatic assay), population doubling time (Trypan blue dye exclusion), migratory ability (wound healing assay) and resistance to a carcinogen (hexavalent chromium (Cr(VI); MTT colorimetric assay and clonogenic assay).

Results: Upon repeated passaging, the morphology and pattern of growth of BEAS-2B cells changed noticeably at ca. passage 30. These changes lasted for another ca. 30 passages, at which time they were gradually reversed (at least partially). Cell size appeared to increase over time in culture, but cellular protein content was unchanged. Based on the observed changes, three passage stages were defined: low, transitional and high. Intracellular Hsp90α protein levels and rates of lactate production increased significantly when cultures reached the transitional stage, but, once again, these changes were partially reversed upon reaching the high passage stage. Population doubling times, on the other hand, decreased steadily over time in culture. No significant changes were observed in terms of migratory ability and resistance to Cr(VI).

Conclusions: Here we show that their repeated subculture for extended periods, such as required for the study of carcinogenesis and other long-term processes, produced several changes that are suggestive of alterations in transformation degree and that may influence experimental outcomes.

56ASM-0227 | Meta-analysis of the effects of exercise-conditioned human serum on the viability of human cancer cell cultures

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Background: Numerous epidemiological studies provided evidence for a link between higher levels of physical activity and lower risk of many types of cancer. Physical activity may lower cancer risk through weight maintenance, by reducing estrogen and insulin levels and by keeping a healthy immune system. There is also some indication from *in vitro* studies that exercise-conditioned human serum affects the viability of cancer cells. The aim of this systematic review with meta-analysis (SRM) was to estimate the magnitude of this effect.

Materials and Methods: Following the PRISMA guidelines and the TREND statement to assess the quality of information (QoI) in each study, nine in vitro studies were included in the present SRM. These studies employed a total of nine human cancer cell lines and serum from 244 individuals (namely healthy sedentary individuals, at risk of prostate cancer individuals and cancer patients), with ages ranging from 18 to 73 years and from six different countries. The impact of acute exercise-conditioned human serum on the viability of cancer cell cultures was analyzed by a variety of assays, such as trypan blue dye exclusion, soft agar assay and the clonogenic assay. Pre-exercise human serum was used for comparison purposes.

Results: Globally, cultures of cancer cell lines exposed to human serum conditioned by acute exercise of various intensities exhibited a reduced viability (compared with control cultures), with an overall effect size (ES) of –1.126 (95% CI; –1.300 to –0.952; $p < 0.001$). The reduction became more pronounced when only those sera conditioned by acute high-intensity exercise were included in the analysis (ES of –1.350 (95% CI;–1.522 to –1.179; $p < 0.001$)).

Conclusions: Our analysis showed that exercise-condition human serum reduced cancer cell viability and that the reduction depended on exercise intensity. Thus, systemic adaptations may be an additional way through which physical activity lowers cancer risk.

56ASM-0229 | Impact of hexavalent chromium, a lung carcinogen, on the stress response of cultured human lung carcinoma cells and on their susceptibility to stress

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Background: Cells exposed to hexavalent chromium [Cr(VI)], an occupational lung carcinogen, experience proteotoxic, genotoxic, oxidative and other stresses. We hypothesized that these stresses activate the stress

response, enabling cells that survive Cr(VI) exposure to withstand further stresses, namely those found in the tumor microenvironment. To test this hypothesis, we evaluated whether exposure to Cr(VI) affected the stress response of cultured human lung carcinoma cells (A549 cells) and their susceptibility to stress.

Materials and Methods: A549 cells were exposed to Cr(VI) for both short (acute exposure) and extended periods of time (chronic exposure). Intracellular Hsp90α protein levels, used to gauge stress response status, were measured by ELISA. Acute heat shock, serum deprivation and nutrient (glucose) deprivation were used to induce stress. The effects of these shocks were assessed in terms of dehydrogenase activity (MTT assay), plating efficiency (clonogenic assay) and trypan blue dye exclusion. The morphology and growth pattern and the population doubling times were monitored (light microscopy and MTT assay) to assess transformation degree.

Results: Preliminary results showed a small increase in intracellular Hsp90α protein levels upon Cr(VI) exposure. In terms of stress resistance, acute Cr(VI) exposure conferred some resistance against heat shock, whereas chronic exposure had the opposite effect. Regarding the other two stressors, chronic exposure to Cr(VI) did not confer protection against partial or total serum deprivation, but conferred some resistance to glucose deprivation. Over the whole duration of the chronic exposure, the morphology and growth pattern did not change, whereas the population of doubling time decreased slightly. This decrease suggests that cells underwent further transformation.

Conclusions: There is some indication that Cr(VI) exposure activated the stress response, but this needs confirmation. Regarding resistance to stress, our results suggest that the impact of Cr(VI) is dependent on both the duration of exposure and type of stressor.

56ASM-0239 | Cytotoxic effect of ridolfia Segetum (L.) moris essential oil against glioblastoma cells

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Background: The medicinal properties of the essential oil (EO) extracted from *Ridolfia segetum* (L.) Moris (*R. segetum*) has attracted the attention of researchers towards ethnomedicine, mainly due to its antioxidant, anti-inflammatory and antitumor effects. Therefore, exploring the antitumor activity of *R. segetum* EO against glioblastoma (GBM), a malignant and incurable tumor, is a viable therapeutic strategy to improve patients' survival.

Materials and Methods: EO was extracted from *R. segetum* according to the protocol of the European Pharmacopeia. The antiproliferative/cytotoxic effect of *R. segetum* EO was assessed in a panel of 5-glioma cell lines (A172, U87, H4, U118 and U373) and in a non-tumoral cell line (HEK293). Briefly, cell viability was assessed by Alamar Blue assay, cell death and cell cycle regulation were analyzed by flow cytometry, using Annexin V/PI and RNase/PI to stain the cells, respectively.

Results: Data obtained showed that 24 hours after treatment, the higher concentration (1 μL/mL) of *R. segetum* EO was able to significantly reduce the viability of tumoral cells (U87) by 60%, while did not induce any significant effect in the non-tumoral cell line (HEK293). Moreover, this cytotoxic effect was also supported by the alterations in the morphology of cells after treatment. Regarding this, 1 μL/mL of this EO promoted cell cycle arrest at G0/G1 phase and induced late apoptosis/necrosis in GBM cells.
Conclusions: Overall, *R. segetum* EO seems to present a selective antiproliferative/cytotoxic activity against tumoral cells, supporting its further exploitation for future therapeutic strategies for GBM.
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56ASM-0241 | Parvifloron D-based therapy for glioblastoma: targeting tumor microenvironment with a natural drug lead

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Background: Glioblastoma (GBM), the most malignant and frequent primary tumor of the Central Nervous System, has a reduced survival rate within 5 years (~14-18 months after diagnosis) due to the lack of effective treatment options and poor prognosis. Concerning that, novel therapeutic approaches based on natural drug leads are an attractive starting point to improve patients' survival rate.
Materials and Methods: Firstly, Parvifloron D (ParvD), a natural drug lead, was isolated from the acetonic extract of *Plectranthus hadiensis* var. *tomentosus* (Benth.) Codd. The antitumoral activity of ParvD was assessed in a panel of 5-glioma cell lines (A172, U87, H4, U118 and U373). Briefly, cell viability was assessed by Alamar Blue assay,

cell death and cell cycle regulation were analyzed by flow cytometry and measurement of apoptosis-related genes expression was performed by qPCR.

Results: ParvD was able to inhibit proliferation, induce cell cycle arrest at G2/M phase and caspase-dependent and -independent apoptosis in the glioma tumor cell panel. Moreover, the necessary dose of ParvD (6.41 μg/mL) to induce inhibition of proliferation by 50% in GBM cells was substantially lower than the temozolomide (first-line treatment) dose (71.41 μg/mL) required to promote the same effect.

Conclusions: Therefore, the outcome of this work has the potential to reduce the multidrug resistance-related to this tumor and contribute to the pursue of hopeful treatments based on ParvD as a drug lead in future chemotherapeutic perspectives for GBM.

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56ASM-0234 | Bioenergetic fingerprinting with coupling and pathway control of coenzyme Q redox state and respiration in permeabilized HEK 293T cells

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Background: Multiple mitochondrial electron transfer (ET) pathways converge at the Q-junction and reduce coenzyme Q, which is oxidized by downstream Complexes III and IV. Diagnostic fingerprinting is challenged with targeting specific segments of pathway and coupling control of oxidative phosphorylation (OXPHOS) [1].

Materials and Methods: We characterised the Q redox state in pathway and coupling control using the Oroboros NextGen-O2k with the electrochemical Q-Module [2], monitoring simultaneously respiration and the reduced Q-fraction in permeabilized HEK 293T cells. Multiple combinations of substrates (pyruvate, malate, succinate) and inhibitors (rotenone, malonate) were used to interrogate the NADH-pathway (N), succinate-pathway (S), or their combination (NS). Coupling control was analysed in the S-pathway (succinate and rotenone), varying from LEAK respiration to OXPHOS-, and ET-capacity.

Results: The reduced Q-fraction increased proportionally with increasing respiration, from N-, S-, and NS-pathway control. This reflects the variable ET push upstream of the Q-junction. In coupling control, the reduced Q-fraction decreased with an increase of respiration, opposite to pathway control. This is caused by the pull effect of coupling control downstream of the Q-junction: Stimulation of respiration by ADP increases the pull and Q becomes more oxidized.

Conclusions: Combined measurements of respiration and the Q-redox state provide unique diagnostic fingerprinting approaches for in-depth diagnostics of mitochondrial function in health and disease.

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56ASM-0235 | Quality control of mitochondrial respiration medium in high-resolution respirometry with living and permeabilized cells

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Background: Quality control is required in clinical studies to ensure accuracy and reproducibility. The technical repeatability of subsamples reflects instrumental resolution and experimental precision, but is insufficiently reported in mitochondrial respiratory research. A frequently neglected problem is quality control of chemicals and incubation media. Mitochondrial respiration medium MiR05 is prepared from MiR05-Kit (Oroboros Instruments), which contains 7 chemicals and is stored as a crystalline powder. The powder is dissolved in H_2O, bovine serum albumin (BSA) is added, and the pH is adjusted.

Materials and Methods: We evaluated the quality of 5 lots of MiR05-Kit stored at room temperature for 2 months to 3 years. Two substrate-uncoupler-inhibitor titration (SUIT) reference protocols [1] were applied in the Oroboros O2k with cryopreserved HEK293T cells. Compared to the instrumental limit of detection of volume-specific O_2 flux of ± 1 pmol·s^{-1}·mL^{-1}, the range of technical repeats in ROUTINE respiration was ± 5 pmol·s^{-1}·mL^{-1} or ± 15 % using 8-16 O2k chambers in parallel at a cell concentration of 10^6 x·mL^{-1}. The biological variability between cell batches was higher. Homogenous stocks of suspended cells were used to compare MiR05 lots at 3 annual intervals.

Results: The MiR05-Kit was stable up to 3 years as shown by consistent cell respiration with different MiR05 lots. Since many different pathway and coupling control states of living and permeabilized cells were covered in the 2 SUIT protocols [1], these results exclude specific inhibitory modifications of MiR05 during storage.

Conclusions: Our study demonstrates a constant quality of MiR05-Kit up to 3 years of storage. This represents an important quality control in high-resolution respirometry to obtain reliable and reproducible results in scientific and clinical investigations.

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Proteomic approach to the characterization of unknown origin male infertility

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Infertility affects around 50 million couples worldwide, with male infertility contributing to half of all cases. When no clear causes for infertility can be found, it is designated of unknown origin (UOMI) that includes the idiopathic (ID) and unexplained male infertility (UMI). Diagnosis of male infertility is firstly based on routine semen analysis, an evaluation with poor prognostic value, stressing the need to deepen the analysis of sperm functionality in these patients to clarify what might be behind their infertility state.

To clarify this issue, we focused on a detailed characterization of sperm function, that goes far beyond the conventional analysis. Functional and bioenergetic parameters such as sperm capacitation, acrosome integrity, chromatin status, and mitochondrial functionality were analysed together with a comprehensive sperm proteomic analysis (SWATH-MS). Furthermore, lifestyle aspects and the symptoms of anxiety and depression were assessed by proper surveys.

Overall, we observed that ID patients besides having significantly decreased sperm concentration, motility and morphology, also presented decreased sperm viability, chromatin integrity and percentage of capacitated cells, comparing to healthy men, with the proteomic results further supporting these results. Moreover, they also had significantly increased incidence of urogenital infections and varicocele. Regarding UMI patients, these were observed to have increased incidence of diagnosed depression, when comparing to the other groups, while in terms of sperm functionality, thought significantly different from ID patients, they showed similarities to that of the healthy control individuals, which was also mirrored at the proteomic level. Finally, 7 important proteins were found, for the first time, to significantly differentiate the 3 patient groups, hence being good candidates for further studies on UOMI.

Overall, this study entailing a unique complete and integrated analysis of the sperm function and proteome from 3 groups of individuals accurately categorized, provided new insights and add knowledge on these patients' infertility unknown aetiology.

Keywords: Unknown origin male infertility, spermatozoa, proteomics

SYMPOSIUM 3: CARDIOVASCULAR & METABOLIC DISEASES

56ASM-0166 | PCSK9's role in cholesterol uptake in different liver cell lines: a specific function in hepatic stellate cells

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Background: The interaction between PCSK9 and LDLR is a well-established regulatory mechanism that controls the clearance of LDL particles from blood; however, the role that PCSK9 exerts on other family members of the LDLR superfamily that also participate in cholesterol metabolism remains to be characterized.
Materials and Methods: We determined the plasma lipid profile and the cholesterol content in livers of Wildtype (*Wt*) and LRP5 knock-out ($Lrp5^{-/-}$) mice fed a normocholesterolemic (NC) or a hypercholesterolemic (HC) diet. We also analyzed the differential expression of cholesterol related genes and proteins including LRP5, PCSK9, VLDLR, LRP6, LRP2 and LRP1. Lipid uptake was studied in liver resident cells (HepG2) and in liver fat storing cells (hepatic stellate cells) with and without LRP5 and PCSK9.
Results: *Wt* HC mice accumulate cholesteryl esters in liver by a mechanism dependent on VLDLR, LRP2, LRP5 and LRP6, as their expression levels are increased compared to *Wt* NC mice. In contrast, in $Lrp5^{-/-}$ HC mice, cholesterol uptake is mainly carried out by scavenger receptors. We also demonstrate that LRP5 and PCSK9 form a complex in the cytoplasm of hepatic stellate cells, but not in HepG2 cells, leading to enhanced lipid uptake in hepatic stellate cells. Furthermore, silencing of PCSK9 or LRP5 significantly reduces lipid uptake in fat-storing stellate cells. Therefore, PCSK9 and LRP5 exert different roles in different liver cell lines.
Conclusions: Our results show that cholesteryl esters accumulate in livers of *Wt* and $Lrp5^{-/-}$ mice. This accumulation can be explained by the increased expression of LRP receptors in HC *Wt* mice or scavenger receptors in HC $Lrp5^{-/-}$ mice. More importantly, we show that PCSK9 and LRP5 bind intracellularly in fat storing liver cells but not in structural liver cells and that both proteins are needed for lipid uptake.

56ASM-0133 | A new crosstalk between dopaminergic and ghrelin/NPY signalling in white adipose tissue as a potential therapeutic target in obesity

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Background: Dopamine counteracts ghrelin's effect in the hypothalamic expression of neuropeptide Y (NPY), which orexigenic effect is linked with major adaptation of white adipose tissue (WAT) for energy storage. We have previously shown that dopamine modulates glucose uptake and catabolic activity in WAT, but its role in regulating WAT NPY is unknown. Our hypothesis is that dopamine regulates WAT ghrelin/NPY system, coordinating peripheral energy balance and WAT function according to the homeostatic needs. As deep perturbations of energy balance, we hypothesized that such mechanisms are impaired in human obesity and consequent metabolic dysregulation.
Materials and Methods: Diet-induced obese type 2 diabetic Goto-Kakizaki rats were treated with bromocriptine (dopamine receptor agonist) for 4 weeks (10mg/kg/day, 6m.o.) and WAT was collected for analysis of ghrelin and NPY machinery. RT-PCR was performed to characterize alterations of WAT dopamine and ghrelin/NPY system from patients with obesity, at different stages of metabolic dysregulation.
Results: Bromocriptine treatment increases GHSR1a and NPY1R protein levels, while improving the metabolic profile of obese diabetic rats. In human WAT, insulin resistance leads to the downregulation of D1 and D4 dopamine receptors, and NPY2R. NPY1R and NPY5R are upregulated in patients with pre-diabetes, decreasing then upon type 2 diabetes establishment. D1R shows a moderate positive correlation with NPY2R ($r = 0.688$, $p < 0.001$) and GHSR1a ($r = 0.727$, $p < 0.001$). Interestingly, a dichotomous association of these receptors was found regarding metabolic/energy balance markers. D1R, GHSR and NPY2R are highly positively associated with UCP1 ($r = 0.850$, $r = 0.702$, $r = 0.711$, respectively, and $p < 0.001$), while NYP1R and NPY5R are correlated with PPARG ($r = 0.679$, $r = 0.779$, respectively, and $p < 0.001$).

Conclusions: We demonstrate, for the first time, the crosstalk of dopamine signalling with WAT ghrelin/NPY machinery, and a dichotomous action of D1R/GHSR1a/NPY2R versus NPY1R/NPY5R receptors in regulating energy balance. Given the upregulation of ghrelin/NPY machinery by bromocriptine, such mechanisms may be a promising therapeutic target in obesity and consequent metabolic sequelae.

56ASM-0149 | Early assessment of bone–related cytokine predicts metabolic response to bariatric surgery

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Background: Emerging evidence are highlighting that bone is an endocrine organ and suggest a complex crosstalk with other metabolic and cardiovascular tissues. Here we investigate the role of two bone-related cytokines (i.e., sclerostin and osteopontin [OPN]) as potential driver of metabolic response after bariatric surgery.
Materials and Methods: A short-term follow-up (1 year) cohort enrolling 94 obese patients undergoing sleeve gastrectomy was used for sclerostin investigation. OPN was instead investigated in a smaller cohort of 41 diabetic patients but longer followed-up (up to three years) treated with biliopancreatic diversion. Anthropometric measure and biochemical values including those related to insulin resistance (HOMA2-IR) β-cell function (HOMA2-%β), and insulin sensitivity (HOMA2-%S) were collected at baseline and during follow-up. Enzyme-linked immunosorbent assay (ELISA) was used for biomarker assay.
Results: Sclerostin but not OPN significantly correlated with glycemic profile at baseline. Reduction of anthropometric indexes was massive in both the cohorts ($p < 0.001$ for all parameters) and associated with a significant improvement in insulin sensitivity. Although sclerostin and OPN significantly decreased during follow-up ($p < 0.001$ for both) the higher the baseline levels of bone-related biomarkers the greater improvement in glycemic profile. This association was tighter with HOMA2-%S. Sclerostin was independent predictor of increase in peripheral insulin sensitivity at 1 year with an adjusted OR of 1.01 (95% CI of 1.01 to 1.02) and a *p*-value of 0.024. Taking advantage of a longer follow-up, OPN was tested as predictor of long-term (3 years) remission form diabetes, showing an adjusted OR of 1.05 (95% CI of 1.01 to 1.10) and a *p*-value of 0.035.
Conclusions: Our data indicate for the bon-related cytokines a role in metabolic improvement after bariatric surgery. Although unpowered and lacking of a clear pathophysiological explanation, these preliminary findings call the attention toward the cross-talk between bone, the surrounding skeletal muscle and the whole metabolic homeostasis.

56ASM-0148 | Circulating exosomal biomarkers in patients with coronary artery disease and metabolic syndrome

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Background: Emerging data showed that circulation exosomal microRNA (miRNA) has been connected with several pathological conditions. Prime objectives of the study to investigate possible role of circulating miRNA subtypes (miR-149-5p and miR-32-5p) in patients with coronary artery disease (CAD) and metabolic syndrome (MetS).
Materials and Methods: Thirty-six patients with CAD were enrolled in this study (Aged 37-73 years; Mean age- 52.20 ± 12.0 years; Male-53%). Patients were divided into two groups by the presence or absence of MetS. Group I – 18 patients with CAD and MetS, whereas Group II -18 patients with only CAD. All anthropometric, laboratory and instrumental data were obtained at baseline for the analysis. Exosomal miRNA were assessed in the serum blood samples according to manufactures instruction. After obtaining and isolating exosomes, protein concentrations were measured by BCA method. And finally four miRNA were detected by real time polymerize chain reactions. All statistical analysis were performed by STATA software.
Results: Exosome protein concentrations were similar in two groups (0.63 ± 0.12 ng/mL vs. 0.67 ± 0.18 ng/mL,

$P > 0.05$). Expression of miR-149-5p and miR-32-5p were significantly higher in patients with CAD with MetS than without MetS group ($P < 0.05$). Among MetS components abdominal obesity (1.3, 1.18-1.65, CI 95%, $P < 0.05$), insulin resistance (1.26, 1.11-1.59, CI 95%, $P < 0.05$) and dyslipidemia (1.15, 1.04-1.45, CI 95%, $P < 0.05$) were positively associated with increased circulating level of miR-149-5p whereas only abdominal obesity (1.21, 1.13-1.55, CI 95%, $P < 0.05$) and dyslipidemia 1.14, 1.05-1.51, CI 95%, $P < 0.05$ were positively associated with increased level of circulating miR-32-5p in patients with CAD.

Conclusions: Circulating exosomal miR-149-5p and miR-32-5p might be prognostic biomarker in patients with CAD and MetS.

56ASM-0006 | Multicistronic plasmids encoding VEGF and FGF2 stimulate local angiogenic processes in vivo: in a matrigel plug assay and a rat model of hind limb ischemia

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Background: Peripheral arteries disease (PAD) is characterized by poor blood supply, blockage of blood vessels, which may ultimately lead to limb amputation. The presence of additional vascular disease and lack of suitable vessels for bypass surgery limits the effectiveness of surgical and physiological treatments. Gene therapy with angiogenic factors, which restores the blood supply to the limb, is a promising alternative to conservative treatment methods.

Materials and Methods: In this study, we developed plasmid constructs that provide simultaneous overexpression genes of vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF2), and reporter protein (DsRed). We applied the system of 2A-peptides of picornaviruses supplemented with a cleavage site by furin proteinase (Fu-2A) to achieve co-expression of genes.

Results: First, we showed high expression and secretion of recombinant VEGF and FGF2 proteins in imported (HEK-293T) and primary cells (HUVEC) *in vitro*. Luminex-Based Multiplex Assay showed that overexpression of VEGF and FGF2 did not affect the secretion of other cytokines and growth factors by both cell types. When transplanting transfected HEK-293 cells, as part of Matrigel plaques, into immunocompromised mice, the expression of recombinant proteins VEGF and FGF2 activated vascular formation processes and also promoted endothelial cell recruitment. Next, we injected the synthesized plasmid constructs into ischemic rat muscle with a two-step surgical model of ischemia induction. At this stage, we observed a gradual decrease in VEGF and FGF2 transgenes expression in the ischemic muscle on 14 and 21 days. Despite this, quantitative analysis of the limb perfusion ratio demonstrated a significant restoration of blood flow on day 21 in the experimental groups. Histological methods showed decreased fibrosis and increased capillary density at 14 and 21 days after injection.

Conclusions: Thus, our study demonstrates the functionality of the constructed plasmid constructs *in vitro* and *in vivo*. Expression of recombinant VEGF and FGF2 proteins as part of multicistronic constructs activated local angiogenesis and recruitment of various cell types. These results suggest the possibility of using this gene therapy construct to stimulate angiogenesis and treat PAD, however, further studies using more representative experimental models and clinical trials are needed.

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56ASM-0008 | Functional properties of langendorff-isolated rat heart recovery after hypodynamia

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Background: The study of the influence of motor activity limitation on the body is an urgent problem of physiology. Restriction of motor activity (hypodynamia) causes changes in contractile function and weakening of the heart muscle, as well as weakening of venous and arterial vessels. We studied the features of rat heart-isolated work according to Langendorff in the recovery period after 30 days of hypodynamia.

Materials and Methods: Restrictions of motor activity were achieved by placing rats in cell cages: the first two days, the time of inactivity was 1 hour, and then increased by 2 hours every 2 days. By day 25, the time spent by animals in the cage-cases reached 23 hours. The parameters of an isolated heart were recorded immediately after physical inactivity, as well as after 2 weeks of the recovery period after 30-day hypodynamia. HR, left ventricular developed pressure (LVP), and coronary flow (CF) were calculated. The recording was carried out on the PowerLab 8/35 apparatus (ADInstruments) using the LabChart Pro software.

Results: During the recovery period, there was an increase of isolated heart LVP by 32.4% ($p < 0.05$), and decrease in heart rate by 6.3% and a decrease in CP by 39.4% ($p < 0.01$) compared with the 30- day hypodynamia.
Conclusions: Recovery after limitation of motor activity is accompanied by an increase in cardiac contraction force (LVP), and a decrease in heart rate and coronary flow. The results can be associated with recovery processes in the heart, since immediately after hypokinesia there was a general tendency to heart activity desadaptation, the force contraction decrease and increase of heart rate. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0009 | Influence of Alpha1(A)–adrenoreceptors stimulation on isolated rat heart coronary flow in ontogenesis

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Background: In the mammalian heart, alpha1-adrenergic receptors (α_1-AR) perform many functions: they participate in the regulation of myocardial contractility, heart chronotropy, coronary blood flow, as well as in various pathological processes. Earlier, we showed that non-selective stimulation of α_1-AR in adult rats reduces coronary circulation, and in 1-week-old rat pups it enhances it. According to scientists, the α_{1A}-AR can mediate a positive inotropic effect in stressful and pathological situations. In this regard, the study of the role of this receptor subtype in the regulation of the blood supply to the heart is gaining relevance. The aim of this study was to investigate the effect of α_{1A}-adrenoreceptors stimulation on coronary flow in isolated hearts of rats in ontogenesis
Materials and Methods: Isolated hearts were perfused in the Langendorff system (ADInstruments). The experiments we used selective agonist α_{1A}-AR A-61603 (10^{-9}mol/L). The degree of coronary circulation was evaluated using the indicator-coronary flow (CF), which was calculated by measuring the amount of perfusate flowing through the coronary vessels of the isolated heart for 1 minute. Statistical processing of the obtained results was performed using the Student's *t*-test.
Results: Perfusion of A-61603 10^{-9}mol/L caused an increase CF isolated heart of 20- and 6-week-old rats by 12% ($p < 0.01$), and 10% ($p < 0.05$), respectively. The speed of isolated heart CF in 3 and 1 week old rats did not change in response to A-61603.
Conclusions: Stimulation of α_{1A}-AR with A-61603 changed the CF in rats of 6 week-old, as well as in adult animals. The absence of changes in CF in 1-week and 3-week-old rats on the introduction of A-61603 is probably due to the lack of sympathetic innervation of the heart of animals at this period of postnatal development. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0010 | Stimulation of α2-adrenergic receptors change heart rate and coronary flow in the newborn rats isolated heart

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Background: Controversial data on the role of alpha2-adrenergic receptors (α_2-AR) in the regulation of coronary blood supply are presented in the modern literature. It was reported that α_2-AR agonists exert a vasoconstrictor effect. On the other hand, the results of the vasodilator effect on coronary vessels of the α_2-AR agonist clonidine hydrochloride are presented in the studies. It is known that α_2-AR is present on the myocardiocytes membranes, and in vascular smooth muscles. The aim was to investigate the effect of different concentrations of the α_2-AR agonist on coronary flow (CF) and heart rate (HR) in the isolated heart of newborn rats.
Materials and Methods: Experiments were performed on 28 newborn rat, they do not have adrenergic innervation of the heart. Isolated hearts were perfused in a Krebs-Henseleit solution - Langendorff (ADInstruments) installation. The CF and HR were calculated. The signals were recorded in a PowerLab system. 10^{-9}–10^{-6}M concentrations range of clonidine hydrochloride (Sigma) were used for the stimulated of α_2-AR. The data were processed statistically using Microsoft Excel software and Student's t test.
Results: Application to a perfuse solution of clonidine hydrochloride (10^{-9}M) decreased CF and causes tachycardia in the newborn rat isolated heart. The α_2-AR agonist (10^{-8}M) caused decreased CF and bradycardia was observed. Clonidine hydrochloride (10^{-7}M) had a different effects CF and HR. The α_2-AR agonist (10^{-6}M) had no effect on the CF, and caused a different effects HR.
Conclusions: Stimulation of alpha2-AR with low concentrations of clonidine hydrochloride (10-9, 10-8 M) leads to a decrease in coronary flow and bradycardia of the newborn rats isolated heart. High concentrations of the α2-AR agonist (10-7, 10-6 M) resulted in opposite

dynamics of heart rate and coronary flow in the newborn rats isolated heart. The study was supported by Russian Science Foundation (grant No. 21-15-00121, https://rscf.ru/ project/21-15-00121/)

56ASM-0011 | Influence of clonidine hydrochloride on the effect of if blockade on isolated rat heart

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Background: Sympathetic control of heart rate plays an important role in the pathophysiology of arrhythmias, hypertension, coronary heart disease, and chronic heart failure. Alpha$_2$-adrenergic receptors (α_2-AR) and hyperpolarization-activated currents (If) are involved in the regulation of heart function. The aim of this study was to investigate the effect of clonidine hydrochloride after of preliminary blockade of If-currents on isolated by Langendorff rat heart.
Materials and Methods: Experiments were carried out ex vivo on isolated hearts of 3-week-old rats ($n = 14$). This age is characterized by significant properties of the heart function associated with the formation of adrenergic innervation. During the experiment, an electrogram of the heart was recorded using atraumatic electrodes. Changes in heart rate (HR) and coronary flow (CF) were recorded after application of the If blocker ZD7288 (10^{-9}mol/L and 10^{-5}mol/L) and the α_2-AR agonist clonidine hydrochloride (10^{-6}mol/L).The data were statistically processed using Student's t-test.
Results: Stimulation of α_2-AR by clonidine hydrochloride after If blockade by ZD7288 (10^{-9}mol/L) in isolated heart of 3-week-old rats increased the HR decline by 20% ($p < 0.01$) and increased CF by 15% ($p < 0.01$). ZD7288 in concentration 10^{-5} mol/L decrease the effect of bradycardia after the application of clonidine hydrochloride by 12% ($p < 0.01$).
Conclusions: Thus, in experiments to studying the role of α_2-AR and If in regulation 3-week-old rats isolated heart was shown that preliminary If blockade enhanced the bradycardic effect and increased blood supply in the isolated heart. The study was supported by Russian Science Foundation (grant No. 21-15-00121, https://rscf.ru/ project/21-15-00121/)

56ASM-0012 | Isolated rat heart function after new cardioplegic solution perfusion

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Background: Cardioplegic heart failure is the most popular method of providing open-heart surgery. Negative changes of ischemia and reperfusion are reduced by quality cardioplegic protection. There is no consensus what types of cardioplegic solutions (CPS) is better. Unfortunately, studies of various cardioplegic solutions are carried out on different experimental models, which makes difficult comparison them with each other. The aim of our study was to evaluate the efficacy of created in Kazan Federal University new extracellular crystalloid CPS in the experiments on isolated rat heart model.
Materials and Methods: Isolated hearts were perfused on a Langendorff apparatus (ADInstruments) with an oxygenated Krebs-Henseleit solution (KH) (37°C, pH = 7.3–7.4) at a constant pressure of 80-82 mmHg. After stabilization of the heart activity, the initial values were recorded. The work was performed according to the following protocol: new solution was administered for 3 minutes, then ischemia was prolonged for 20 minutes, then the heart perfusion was resumed with KH solution. The heart rate was recorded during 40 minutes of reperfusion. The assessment of the contractility of the myocardium was carried out according to the indicator of left ventricular developed pressure (LVDP). The signals were recorded on the PowerLab 8/35 setup using the "LabChart Pro" program. Statistical processing of the obtained results was carried out using the Student's t-test.
Results: Asystole was achieved within 1 minute of CPS administration. Recovery of spontaneous cardiac activity after myocardial ischemia induced by the new CPS occurred within the first minute of reperfusion in 100% cases. Decrease in myocardial contractility compared to the initial values was not observed during the entire reperfusion period ($LVDP_{initial} = 52 \pm 5.2$ mmHg and $LVDP_{reperfusion} = 58 \pm 5.8$ mmHg), what allows us to conclude about the effectiveness of myocardial protection by the new CPS.
Conclusions: In our experiment on a model of an isolated rat heart, which is widely used for the study of various CPR, we showed that the new solution is able to quickly and effectively cause myocardial plegia, and also does not interfere with the rapid and full recovery of its function after the start of reperfusion. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0013 | The role of NPY-receptors on generation of the action potential of newborn rats cardiomyocytes

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Background: Neuropeptide Y (NPY) is a 36 amino acid peptide isolated from the pig's brain that is synthesized and released from the sympathetic nerves and adrenal medulla. NPY has been identified in the tissues of many animal species. The aim of this work was to study the role of neuropeptide Y in the regulation of electrical activity of the myocardium of the right atrium and ventricle of rats in early postnatal ontogenesis.
Materials and Methods: The study was carried out on rats ($n = 32$). Membrane potential (MP) and action potential (AP) were recorded using glass microelectrodes. The stimulus duration (1ms) and repetition rate (3Hz). Statistical significance was assessed using Student's t-test.
Results: NPY at a concentration of 10^{-10}, 10^{-9} and 10^{-6}mol/L did not cause significant changes in MP and AP parameters of atrial and ventricular cardiomyocytes in newborn animals. NPY at a concentration of 10^{-8}mol/L shortened the phase of repolarization of AP of working atrial cardiomyocytes. By the 7th minute, NPY reduced APD20 by 28% APD50 by 33% APD 90 by 35%($P < 0.05$). NPY at a concentration of 10^{-8}mol/L shortened the phase of repolarization of AP of working ventricle cardiomyocytes. By the 7th minute, NPY reduced only APD90 by 14% ($P < 0.05$). NPY at a concentration of 10^{-7}mol/L slightly shortening the phase of repolarization of AP. By the 7th minute, APD50 is 14% ($P < 0.05$), APD90 is 15% ($P < 0.05$) of working ventricle cardiomyocytes.
Conclusions: Neuropeptide Y causes changes in the pattern of electrical activity of the myocardium of the atria and ventricles. The data obtained by us on a decrease in the APD are most likely related to changes in the density and kinetics of K-channels, which leads to an increase in the total K-current. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0039 | The role of fibrinogen fragments Aα505-610 and 414-610 in interactions with platelets, tumor and endothelial cells

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Background: A complex multidomain protein fibrinogen is the major component of the blood clotting system. In addition to its function in blood clotting fibrinogen also plays a crucial role in wound healing by promoting physiological inflammation and angiogenesis and may contribute to pathological process via cells interactions. The aim of our work was to estimate the role of different sites of BβN- and αC-domains of fibrin(ogen) in aggregation of platelets, proliferation of endothelial cells and migration of tumor cells.
Materials and Methods: Partly hydrolyzed forms of fibrinogen desAα505-610 and desAα414-610 were obtained by limited proteolysis using specific proteases from *Bacillus thuringiensis* cultural medium and venom of snakes *Calloselasma rhodostoma* and *Echis multisquamatis*, respectively. Fibrinogen-mediated platelet aggregation was assessed by aggregometry. Wound healing test was performed on HeLa cells and migration activity was measured 24 hours after scratch. MTT assay was conducted on mouse aortic endothelial cells (MAEC) to evaluate the role of truncated forms of fibrinogen in maintaining endothelial cell viability.
Results: Decreasing of platelet aggregation levels by 15 % was showed compared to control in the presence of fibrinogen desAα505-610. However, the velocity of aggregation was not changed in the presence of this form. The loss of Aα414-610 fragment by fibrinogen molecule reduced both rate and velocity of aggregation by 60 % and 40 % in comparison with control, respectively. Modification of cultural surface with fibrin desABAα505-610 and desABAα414-610 led to significant decrease of the number of viable endothelial cells in 1.28 times compared to the surface treated with fibrin desAB. At the same time we did not find the significant difference between partially hydrolyzed forms of fibrin. Both forms of impaired fibrinogen affected HeLa migration activity by 25 % in comparison with full-length fibrinogen. No difference of migration area between samples with added desAα505-610 and desAα414-610 forms of fibrinogen was detected.

Conclusions: The loss of the Aα505-610 fragment reduced the ability of fibrinogen to support platelet aggregation. Additional loss of the Aα414-504 fragment exacerbated this effect, reducing both the rate and velocity of platelet aggregation. The Aα414-504 fragment was found to be extremely important for maintaining platelet aggregation. Both Aα414-504 and Aα505-610 fragments were important for proliferation of endothelial cells and migration of tumor cells.

Work was partially supported by the Targeted program of NAS of Ukraine for Young sciences laboratories: "Identification of molecular recognition sites of fibrin(ogen)". The research was carried out in the frame of postdoctoral program of NAS of Ukraine: "The effect of fibrin fragments as a component of the extracellular matrix on cell growth and differentiation".

56ASM-0041 | Negative ultraslow potentials during kidney ischemia

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Background: Negative ultraslow potential (NUP), recently discovered in patients with developing ischemic brain lesions, have attracted the attention as a potential prognostic factor of the outcome. However, it remains unknown whether ischemic NUPs are inherent only to neuronal tissue or they are also presented in non-neuronal tissue.

Materials and Methods: The study was performed on the kidneys of the urethane-anaesthetized Wistar rats *in vivo* ($n = 5$). Electrical signals were recorded from renal cortex using 16-channel iridium microelectrodes. A near-infrared diode positioned under the kidney provided intrinsic optical signals (IOS) recordings. Ischemia was induced by injection of the vasoconstrictor endothelin-1 (ET1, 20 μM) into the renal cortex in the vicinity of the renal artery entry point.

Results: Electrical signals similar in dynamics and amplitude to NUP in the neocortex started within 4.7 ± 2.3 minutes after ET1 injection and displayed similar profile throughout the depth of the renal cortex. NUPs' peak amplitude of -42 ± 7 mV was attained 45.8 ± 18.7 minutes after ET-1 injection followed by a progressive decrease of the amplitude. 60-120 min after the ET-1 injection the extracellular potential returned to the control values. Extracellular voltage shifts were accompanied by biphasic change in the IOS. In the area of ET-1 injection light transmission first increased with maximal transmittance attained 5-20 min after the ET-1 injection followed by a gradual decrease below the control level, and then returned to the control values at the end of the 3rd hour of registration.

Conclusions: Thus, NUPs also develop in non-neuronal tissue during ischemia, and therefore are more general electrical signature of ischemia. Our findings support hypothesis that oxygen-sensitivity of electrodes made of noble metals contributes to NUP.

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56ASM-0076 | Neuropeptide Y as a cardioprotector of sympathetic effects on the heart

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Background: Neuropeptide Y (NPY) ATP is the main mediators of norepinephrine and contained in the same vesicle. It is believed that NPY modulates regulatory influences by being located postsynaptically and presynaptically, inhibiting the release of norepinephrine. In the heart discovered Y1, Y2, Y3, and Y5-receptors. These receptors can be used as potential therapeutic targets. The aim of this study is to study the modulating effect of [Leu31, Pro34] NPY on the bioelectrical parameters of the right atrium with a preserved sinus node and spontaneous activity in adult animals.

Materials and Methods: The study was carried out on laboratory rats of 100-days old ($n = 20$). We studied the electrical activity of the right atrial myocardium with a preserved sinus node and spontaneous activity. We studied the electrical activity of cardiomyocytes using an intracellular microelectrode Statistical significance was carried out using paired Student's t- test. To study the possible influence of [Leu31, Pro34]NPY on the effects of isoproterenol, we added a selective agonist against the background of isoproterenol.

Results: [Leu31, Pro34]NPY at 10^{-7}mol/L attenuates the effects of isoproterenol. The $\beta_{1,2}$-adrenergic agonist isoproterenol 10^{-8}mol/L increases the duration of action potential (AP) by 33% ($p < 0.01$). The application of the agonist NPY1,5-receptor reduces the frequency of AP generation from 33%($p < 0.05$) to 6%. Isoproterenol reduces APD 20, APD 50 and APD 90 by 46%; 45%; 45%, respectively ($p < 0.001$; $n = 10$). The joint application of agonists weakens the effect of isoproterenol on the repolarization duration AP of the atrial myocardium: a decrease in DAP50 to 25% is observed ($p < 0.05$; $n = 10$), APD20, APD90 did not change significantly.

Conclusions: Thus, the combined action of isoproterenol and [Leu31, Pro34]NPY in 100-day-old rats leads to a decrease the effect of isoproterenol on the parameters of electrical activity of the right atrial myocardium.

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56ASM-0080 | The impact of atmospheric chemicals on the cardiovascular system of adolescents

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FOOD Lab Healthy and safe food

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Background: Heart disease remains the leading cause of death worldwide, and deaths in Russia are two times higher than in European countries.

Materials and Methods: Non-carcinogenic risk assessment was carried out based on the results of studies performed on the basis of an accredited laboratory in the Republic of Tatarstan according to the guidelines for risk assessment for public health and the USEPA Environmental Protection Agency.

Results: We have identified the zones of the city of Kazan for research: 1 (Kirov), 2 (Privolzhsky), 3 Sovetsky, 4 Moscow, 5 Vakhitovsky. Under conditions of combined exposure, the main critical target organs experience the greatest toxicological load: respiratory organs (HI = 3.04 - 2.36), CCC (HI = 0.88 - 0.55), blood (HI = 0.84 - 0 ,53), The highest values of the risk of non-carcinogenic effects of disorders affecting the overall development of the body and diseases of the cardiovascular system are almost the same and are observed in HI = 0.63 (1) and HI = 0.62 (2) zones, in other areas the risk was 0 .56 - 0.55. In zones 2-4, the values are close to HI = 0.26 - 0.21.

Conclusions: The results of assessing the total risk of non-carcinogenic effects from chemicals showed that the largest contribution to the total HI value is made by: carbon (soot), nitrogen dioxide, suspended particles PM2.5 and PM10. The list of studied substances does not exceed the threshold of the hazard coefficient, however, their sum revealed high risk values in 2 (HI = 3.56), 1 (HI = 2.41), 4 (HI = 2.10), 3 (HI = 2, 11) and 5 (HI = 2.07) zones. The highest values of the risk of non-carcinogenic effects of diseases in adolescents are characteristic of the respiratory organs and the cardiovascular system.

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56ASM-0316 S3 IS | LOX-1 and cardiovascular disease: pathophysiological and prognostic role

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Cardiovascular diseases are main cause of morbidity and mortality in the Western World. Several human disorders belong to the pool of age-related CVD including atherosclerosis, thrombosis and myocardial infarction (MI). On molecular level large number of genes and their encoded proteins are involved in CVD on different stages. One of them is lectin-like oxidized low-density lipoprotein scavenger receptor (LOX-1). Using endothelial-specific LOX-1 transgenic mouse line we could elucidate the role of LOX-1 in the setup of atherosclerosis, arterial thrombosis and stroke. In the current research projects, we are trying to investigate the age-dependent *in vivo* role of LOX-1 in myocardial infarction, sickle cell disease and chronic kidney disease using genetically modified mouse models in combination with genetic model of SCD and experimental models of myocardial infarction and chronic kidney disease. Furthermore, applying multi-omic approaches on isolated cells, we are going to identify LOX-1-dependent molecular regulators as well as downstream targets.

56ASM-0154 | Involvement of ion channels in heart failure-induced kidney dysfunction: a focus on inflammation

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Background: Heart Failure (HF) is one of major worldwide health-care problem associated with high morbidity and mortality rates. Based on the close relationship between heart and kidney in preserving water and electrolytes homeostasis and the cardio-circulatory function, an impaired heart-kidney cross-talk leads to an acute or chronic mutual dysfunction. Renal ion channels (ClC-K, Kir4.1, Kir5.1) or transporters (ClC-5, NKCC), play a key role for salt and protein reabsorption and may represent targets in HF management. It is well know that inflammation contributes to the development of HF-induced kidney dysfunction. Accordingly, $CD8^+$ T cells infiltration in kidney leads a sodium chloride balance alteration in hypertension (Liu et al., *Nat Comun*. 2017). So far, the link between inflammation components and renal ion channels in the context of HF is unknown. Thus, in this study, we performed an analysis of ion channels expression and activity by *in vivo*/*in vitro* studies.
Materials and Methods: We used Dahl salt-sensitive (SS) hypertensive rats, fed with a high salt diet, for performing gene expression analysis. In parallel, cytokines effect on renal ion channels was tested through heterologous expression in HEK cells and patch clamp recordings.
Results: We detected a significant reduction of ClC-K1, Kir4.1, Kir5.1 and ClC-5 mRNA in kidney of Dahl/SS rats *vs* control rats associated with a high TNF-a, IL-6, TGF-b plasma levels. Importantly, to gain insight into the molecular mechanism regarding renal ion channels and inflammation, we assessed ClC-Ka chloride currents after cytokines incubation. We demonstrated that TNFα and IL-6 induced an increment of chloride currents at concentrations consistent with those detected in the HF animal model under investigation.
Conclusions: Tubular dysfunction and proteinuria observed in HF Dahl/SS rats could be related to altered renal ion channels gene expression in association with cytokines overproduction. Renal ion channels could represent appealing druggable targets in HF (PRIN2017- Prot. 2017NKB2N4).

56ASM-0155 | Entry of nitrates and arsenic with food as a risk factor of developing cardiovascular diseases in residents of tatarstan

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Background: The entry of nitrates (in NO3) and arsenic (As) with foods (F) increases the risk of developing cardiovascular diseases (CVDs), ranking first in the population mortality structure.
Materials and Methods: Calculation of exposure to contaminants was carried out according to the data from the household budget survey
Results: Calculation of exposure to contaminants was carried out according to the data from the household budget survey (HBS) (the upper 95% confidence interval (CI), the intake level of basic food groups (kg/day) and the regional value of an adult's weight (62 kg). A statistical model for NO3 dose during the intake of 100 g of each food group obtained by means of multilinear regression analysis, is the following: S (mg/kg per day) = 7.4-0.14F 1+0.47F2+1.1F3+1.3F4+0.5F5, where F1-body weight, F2-F5-food groups (bread, potatoes, vegetables and cucurbits, fruit and berries). A statistical model for As includes 5 food groups, and apart from those listed above it includes also sugar, meat and dairy products, and eggs (g).
Conclusions: The results of regression analysis showed that the amount of foods in the daily diet (g) and the body weight were the major factors having impact on the level of chemical load with nitrates NO3 and As, and a high risk of developing cardiovascular diseases (CVDs) in the residents of Tatarstan (hazard index HI-8.6).

56ASM-0156 | Efficacy of diet with low glycemic index in atherosclerotic coronary artery disease

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Background: Diet with low glycemic index showed several benefits in reducing atherosclerotic biomarkers in patients with coronary artery disease (CAD). Objectives of the study was to evaluate the efficacy of the diet with low glycemic index in patients with CAD in terms of blood inflammation state and lipid parameters.

Materials and Methods: One hundred and sixty patients aged 38-76 years established with CAD entered as 12 week dietary intervention either with diet with low glycemic index ($n = 80$) or routine diet ($n = 80$) together with standard therapy from 2016 to 2019 (male = 48%; 58.2 ± 12.0 years). Laboratory (including hs-CRP, pro-inflammatory interleukins, IL-1β, IL-6, TNF-α, lipid parameters TC, TG, LDL-Cholesterol, HDL-Cholesterol) and instrumental data were obtained at baseline and in 12 weeks of the intervention.

Results: There were no statistically differences in biochemical data between two groups at their baseline characteristics. Diet with low glycemic index positively influenced on hs-CRP (from 252.4 ± 40.6 mg/dL to 161.9 ± 28.5 mg/dL vs. from 237.8 ± 35.6 mg/dL to 202.4 ± 23.8 mg/dL; $P < 0.05$), HbA1c (from 6.95 ± 1.95 % to 4.78 ± 1.18 % vs. 6.80 ± 1.65 % to 6.25 ± 1.45%; $P < 0.05$), TG (from 5.2 ± 2.2 to 3.1 ± 1.8 vs. from 5.8 ± 2.8 to 4.9 ± 2.0, $P < 0.05$), TNF-α (from 1.48 ± 0.91 to 0.88 ± 0.19 vs. from 1.55 ± 1.35 to 1.12 ± 0.35, $P < 0.05$), IL-6 (from 8.2 pg/mL to 4.9 pg/mL vs. from 8.2 pg/mL to 4.9 pg/mL, $P < 0.005$) than routine diet. Although reduction in IL-1β were observed in both groups (from 32.5 ± 17.2 pg/ml to 28.9 ± 16.8 pg/ml, $P > 0.05$; vs. 33.6 ± 21.6 pg/ml to 29.8 ± 20.4, $P > 0.05$;), however there were no statistically significant from baseline and between groups ($P > 0.05$).

Conclusions: Diet with low glycemic index demonstrated superiority to routine diet to improve inflammatory state and lipid parameters in patients with CAD.

56ASM-0331 | TP and TQ intervals are inversely related to diastolic dysfunction and HFpEF

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Background: Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome characterized by left ventricular diastolic dysfunction (LVDD). The diastolic filling depends on the diastolic interval, and because the QT-interval is prolonged in HF there is a shorter electrical diastole. We hypothesize that a short electrocardiographic diastolic time (the TQ-interval) is related to diastolic dysfunction and HFpEF. Because the QT-interval is longer (and therefore the TQ-interval may be shorter) in women than in men, we also assessed whether this relation was sex-dependent.

Methods: Cross-sectional electronic health record data from Cardiology Centers of the Netherlands between 2011 and 2018 were analyzed. In that period, and in 92,131 patients concurrent electrocardiograms (ECGs), echocardiograms, and corresponding information on diastolic function and heart failure status was obtained. At the first visit in one of the 13 centers, 66% of patients had neither diastolic dysfunction nor HF (n=61,087, control group), 30% had isolated diastolic dysfunction (LVDD, n=27,443), 3% had HFpEF (symptoms of HF, LVEF ≥50%, and diastolic dysfunction, n=2,788), and 1% had HFrEF (n= 813, symptoms of HF, LVEF <50%). We used multivariable logistic regression analyses to study the association between TQ and TP interval (per standard deviation) and diastolic dysfunction and HFpEF, respectively. Interaction with sex was tested.

Results: PR, QRS and QT intervals were longer in patients with diastolic dysfunction and HFpEF compared

to controls. The TP intervals were significantly shorter in the LVDD and HFpEF groups (308 (±SD 126) ms and 313 (±SD 137) ms, respectively) than in the control group (362 (±SD 145) ms) as were the TQ intervals (LVDD group 479 (±SD 130) ms, HFpEF group 482(±SD 141) ms, and control group (519 (±SD 150) ms), respectively. After correction for potential confounders, both the longer TP and TQ intervals remained independently associated with a lower risk of LVDD and HFpEF; for LVDD; adjusted OR= 0.59 (95% CI: 0.55 0.62) and 0.58 (95% CI: 0.54, 0.61), for HFpEF; adjusted OR= 0.67 (95% CI: 0.59, 0.75) and 0.76 (95% CI: 0.67, 0.87) (see Table 1). There was no significant sex-interaction.

Conclusions: Shorter TP and TQ intervals are associated with an increased risk of having LVDD and HFpEF in women and men in outpatient clinics. The strong association between TP and TQ intervals with both diastolic dysfunction and HFpEF implies that electrical abnormalities may precede diastolic dysfunction at least in some patients. However, this needs to be tested in longitudinal studies.

56ASM-0132 | Uric acid induces vascular smooth muscle cell migration through angiotensin II and proteasome

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Background: Growing evidence indicates that high levels of uric acid (UA) are a risk factor for the development of cardio-renal disease because of the involvement in vascular inflammation and remodeling. Vascular smooth muscle cells (VSMCs) are highly plastic cells able to move from a contractile to a secretory state. Whether UA is involved in this VSMC phenotypic transition is not yet known.

Materials and Methods: MOVAS, a mouse VSMC cell line, was exposed to 6, 9 and 12 mg/dL of UA or control for 24-48 hrs. Proliferation and migration were then tested alongside with the effect of atrogin-1 and its suppression by MG132. The involvement of angiotensin II (A2) was finally tested through AT1 Receptor (AT1R) mRNA quantification and its blocking with losartan (L) or valsartan (V) (both at 10 μmol).

Results: UA had no effects on cell viability but small increase of cell proliferation at 24h (+11-15%). Rather, UA promoted the migratory rate at 24h and 48h ($p < 0.001$), confirmed by phalloidin staining. UA also significantly increased smoothelin B, αSMA and SM 22α levels at 24 h (+20/50%), with a subsequent drop at 48h. VSMCs also increase their area (+ 30%) in response to UA stimulation, in a time- and concentration-independent manner. Atrogin-1 was 2-fold up-regulated at 48h after UA exposure ($p = 0.04$) with an inverse correlation between its protein and αSMA expression. MG132 instead blunted the migration and F-actin re-arrangement. Similarly, L and V blocked VSMC migration ($p < 0.001$ vs UA) and finally inhibited UA-induced increase in cells area ($p < 0.001$ vs UA).

Conclusions: Our findings indicate UA as key player in vascular remodeling through detrimental effects on VSMC proliferation, cell area, migration and cytoskeleton. A2 and proteasome inhibitors may rescue VSMC from such a detrimental phenotypic with protective effects on cardio-renal disease induced by UA.

56ASM-0286 S3 IS | Sympathetic nervous system-microbiota axis and cardiovascular function

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Recent evidence highlights the key role played by the intestinal microbiota in both health and many human diseases. Gut microbiota is involved in metabolizing ingested nutrients into fibre-derived short-chain fatty acids (SCFAs), trimethylamine N-oxide (TMAO), secondary and tertiary bile acids (deoxycholic acid, lithocholic acid, ursodeoxycholic acid) from hepatic primary bile acids (cholic acid, chenodeoxycholic acid), and branched amino acids. TMAO has been associated with atherosclerosis and cardiovascular diseases. Nutritional pathways in the gut are under the control of the autonomic nervous system-microbiota axis. These complex pathways are involved in metabolic syndrome and overweight/obesity. We need to unravel the mechanisms through which intestinal microbiota can modify the adipose tissue dysfunction and metabolism causing cardiovascular diseases. Intestinal dysbiosis can shift the production of SCFAs affecting both lipid and glucose metabolism, as well as the status of chronic, low-grade- metabolically-related inflammation. SCFAs are also involved in mechanisms governing the gut barrier and related intestinal permeability. Deranged intestinal permeability paves the way to lipopolysaccharide-mediated endotoxemia, and liver steatosis. Bariatric surgery is a validated therapeutic

option for severe obesity, but this approach inevitably leads to profound changes of intestinal microbiota. Finally, recent data suggest mechanisms by which the gut microbiota mediates gender differences in cardiovascular disease risk.

56ASM-0191 | Impact of diet intervention and rosuvastatin treatment on the gut microbiota of hypercholesterolemic pigs

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Background: Potential involvement of intestinal microbiota in cardiovascular risk has recently gained significant interest. However, the effect of primary prevention strategies including lifestyle and statin intervention on gut microbiota has yet to be addressed. This study investigates the impact of diet and rosuvastatin treatment on gut microbiota of diet-induced hypercholesterolemic pigs.
Materials and Methods: Pigs ($n = 32$) were fed a high-fat diet (HF-D) for 10 days (cholesterol≈400mg/dL). Thereafter, hypercholesterolemic pigs were distributed into four arms of 30-day interventions: I) maintained on HF-D ($n = 9$); II) switch to normocholesterolemic diet (NC-D) ($n = 8$); III) switch to NC-D plus 40mg rosuvastatin/daily ($n = 8$); or IV) maintained on HF-D plus 40mg rosuvastatin/daily ($n = 7$). Feces were collected at study endpoint for phylogenetic and taxonomic characterization of the gut microbiota (16S rRNA sequencing), and functional profile prediction (PICRUSt2). Furthermore, plasma TMAO levels, lipid parameters, and liver and kidney functions were determined.
Results: 30 days of NC-D significantly reduced cholesterol levels. In contrast, rosuvastatin-treated pigs showed no changes in plasma lipid concentrations although liver HMG-CoA reductase activity was significantly reduced ($p < 0.05$). Diet modulated microbiota populations (PERMANOVA, $p = 0.001$) while rosuvastatin exerted no effect. As such, animals that switched to NC-D displayed significantly higher alpha-diversity in comparison to those animals that remained on HF-D ($p = 0.001$). Differential abundance analysis supported these findings and 14 genera differed between diets (ANCOM). PICRUSt2 analysis revealed diet-dependent metabolic capacities of gut microbiota populations. These findings were accompanied by a significant reduction in the harmful metabolite TMAO in NC-D animals versus HC-D pigs ($p < 0.0001$).
Conclusions: Reduction in fat intake is able to modify the composition of gut microbiota in favor of alpha-diversity and towards a healthy metabolic profile, whereas 1-month rosuvastatin intake exerts no effects. These findings evidence the beneficial effect of low-cholesterol diet in modulating the gut microbiota and TMAO production.

56ASM-0169 | Early assessment of metabolic-related biomarkers osteopontin and resistin predicts early mortality in non-small cell lung cancer undergoing treatment with nivolumab

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Background: Although nivolumab has dramatically changed the clinical history of non-small cell lung cancer (NSCLC), the improvement of patient profiling would have relevant clinical implications. By linking with metabolic profile, here we investigate the role of cardiovascular-related cytokines (i.e., resistin and osteopontin).
Materials and Methods: This prospective study enrolled 78 patients with advanced NSCLC enrolled. Blood samples were collected at during the time of the first five nivolumab administration. Serum levels of osteopontin (OPN) and resistin were then assessed by enzyme-linked immunosorbent assay (ELISA). The primary endpoint of the study was to evaluate the predictive value of OPN and resistin towards the overall survival (OS).
Results: At baseline, high serum levels of OPN but not resistin were associated with a worse performance status. OPN values at baseline were also associated with the first and best response according with Response Evaluation Criteria in Solid Tumors (RECIST). OPN and resistin significantly correlated with each other, with C-reactive

protein, blood cell count, neutrophils and as well as their related activation biomarkers: myeloperoxidase, matrix metalloproteinase (MMP)-8 and MMP-9. Over time median level of both OPN and resistin peak at cycle two and then dropped down until the last cycle. Survival analysis revealed a significant predictive ability toward OS for early OPN assay (HR at baseline 3.125 with a 95% CI of 1.41 to 6.94) and resistin (HR at second cycle 2.85 with a 95% CI of 1.22 to 6.67).

Conclusions: Our data indicate for the early assessment of both OPN and resistin a potential role in the outcome of NSCLC treated with nivolumab. Although unpowered and lacking a clear pathophysiological explanation, these preliminary findings call the attention toward the innate immune activation in NSCLC, potentially linked with metabolic profile.

56ASM-0011 | Influence of clonidine hydrochloride on the effect of If blockade on isolated rat heart

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Background: Sympathetic control of heart rate plays an important role in the pathophysiology of arrhythmias, hypertension, coronary heart disease, and chronic heart failure. Alpha$_2$-adrenergic receptors (α_2-AR) and hyperpolarization-activated currents (If) are involved in the regulation of heart function. The aim of this study was to investigate the effect of clonidine hydrochloride after of preliminary blockade of If-currents on isolated by Langendorff rat heart.

Materials and Methods: Experiments were carried out ex vivo on isolated hearts of 3-week-old rats (n=14). This age is characterized by significant properties of the heart function associated with the formation of adrenergic innervation. During the experiment, an electrogram of the heart was recorded using atraumatic electrodes. Changes in heart rate (HR) and coronary flow (CF) were recorded after application of the If blocker ZD7288 (10^{-9}mol/L and 10^{-5}mol/L) and the α_2-AR agonist clonidine hydrochloride (10^{-6}mol/L).The data were statistically processed using Student's t-test.

Results: Stimulation of α_2-AR by clonidine hydrochloride after If blockade by ZD7288 (10^{-9}mol/L) in isolated heart of 3-week-old rats increased the HR decline by 20% ($p < 0.01$) and increased CF by 15% ($p < 0.01$). ZD7288 in concentration 10^{-5} mol/L decrease the effect of bradycardia after the application of clonidine hydrochloride by 12% ($p < 0.01$).

Conclusions: Thus, in experiments to studying the role of α_2-AR and If in regulation 3-week-old rats isolated heart was shown that preliminary If blockade enhanced the bradycardic effect and increased blood supply in the isolated heart. The study was supported by Russian Science Foundation (grant No. 21-15-00121, https://rscf.ru/project/21-15-00121/)

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56ASM-0012 | Isolated rat heart function after new cardioplegic solution perfusion

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Background: Cardioplegic heart failure is the most popular method of providing open-heart surgery. Negative changes of ischemia and reperfusion are reduced by quality cardioplegic protection. There is no consensus what types of cardioplegic solutions (CPS) is better. Unfortunately, studies of various cardioplegic solutions are carried out on different experimental models, which makes difficult comparison them with each other. The aim of our study was to evaluate the efficacy of created in Kazan Federal University new extracellular crystalloid CPS in the experiments on isolated rat heart model.

Materials and Methods: Isolated hearts were perfused on a Langendorff apparatus (ADInstruments) with an oxygenated Krebs-Henseleit solution (KH) (37°C, pH = 7.3-7.4) at a constant pressure of 80-82 mmHg. After stabilization of the heart activity, the initial values were recorded. The work was performed according to the following protocol: new solution was administered for 3 minutes, then ischemia was prolonged for 20 minutes, then the heart perfusion was resumed with KH solution. The heart rate was recorded during 40 minutes of reperfusion. The assessment of the contractility of the myocardium was carried out according to the indicator of left ventricular developed pressure (LVDP). The signals were recorded on the PowerLab 8/35 setup using the "LabChart Pro" program. Statistical processing of the obtained results was carried out using the Student's t-test.

Results: Asystole was achieved within 1 minute of CPS administration. Recovery of spontaneous cardiac activity after myocardial ischemia induced by the new CPS occurred within the first minute of reperfusion in 100%

cases. Decrease in myocardial contractility compared to the initial values was not observed during the entire reperfusion period (LVDPinitial = 52±5.2 mmHg and LVDPreperfusion = 58±5.8 mmHg), what allows us to conclude about the effectiveness of myocardial protection by the new CPS.

Conclusions: In our experiment on a model of an isolated rat heart, which is widely used for the study of various CPR, we showed that the new solution is able to quickly and effectively cause myocardial plegia, and also does not interfere with the rapid and full recovery of its function after the start of reperfusion. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0099 | The effect of α2-adrenoreceptors on the isolated rats heart contractility with a model of myocardial infarction in the acute stage

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Background: Stimulation of α2-adrenergic receptors (α2-AR) has a direct cardioprotective effect. The researchers revealed an increase in the expression of α2-AR subtypes in rats with spontaneous hypertension, as well as the dysfunction of α2-AR and the inefficiency of signaling pathways associated with α2-AR. The aim of this study was to investigate the effect of α2-AR stimulation of the isolated heart contractility with a myocardial infarction (MI) model 24 hours after the influence.

Materials and Methods: Ex vivo experiments were performed on isolated hearts of intact rats ($n = 7$) and rats with a model of MI ($n = 10$) 24 hours after the operation. IM is reproduced by ligation of the anterior branch of the left coronary artery. Agonist α_2-AR clonidine hydrochloride was used in concentration of 10^{-9} Mol. The data were statistically processed using Student's t-test.

Results: A comparative analysis of a_2-AR stimulation revealed that in intact rats, the agonist reduced contractility by 21% ($p < 0.05$), and with the MI model, 24 hours after modeling, it increased by 38% ($p < 0.01$).

Conclusions: Thus, in our experiments α2-AR stimulation revealed multidirectional effects on inotropy reaction of intact rats isolated heart and rats with the MI model. The study was supported by Russian Science Foundation (grant No. 21-15-00121, https://rscf.ru/project/21-15-00121/)

56ASM-0100 | If-currents are involved in heart contractility regulation of rats with acute myocardial infarction model

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Background: Many factors have been identified in the development of coronary heart disease, such as hypertension, diabetes, smoking and an increase in heart rate. "Funny currents" (If-currents) play a key role in the electrogenesis of cardiomyocytes. The density of If currents increase in atrial and ventricular myocytes in patients with heart failure. The aim of this study was to investigate the effect of the blockade If-currents on the contractility of isolated heart with a model of myocardial infarction (MI) 24 hours after experimental influence.

Materials and Methods: Ex vivo experiments were performed on Langendorff-isolated hearts of intact ($n = 7$), sham-operated ($n = 6$) rats and with a model of MI ($n = 10$) 24 hours after experimental influence. IM is reproduced by ligation of the anterior branch of the left coronary artery. To block If currents, ZD7288 was used at a concentration of 10^{-9} Mol. The data were statistically processed using Student's t-test.

Results: Blockade of If-current revealed that in intact rats group ZD7288 increased contractility by 47% ($p < 0.05$), in sham-operated animals – by 23% ($p < 0.01$), in the MI model 24 hours after the simulation increased by 20% ($p < 0.01$).

Conclusions: Thus, our study showed that in the group of rats with a model of acute myocardial infarction, the effect of If on the contractility of the isolated heart decreases. The study was supported by Russian Science Foundation (grant No. 21-15-00121, https://rscf.ru/project/21-15-00121/)

56ASM-0101 | Analysis of rats myocardial contraction force at different stages of experimental infarction

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Background: The study of experimental models of myocardial infarction (MI) is necessary for the development of innovative methods of treatment of this disease. The aim of this study is to conduct a comparative analysis of the

effect of myocardial infarction on the contractility of the isolated rat heart at different stages of MI.

Materials and Methods: The experiments were performed ex vivo on isolated hearts of intact and rats with a model of MI after 1 day, 54 days and 120 days after the simulation. MI was performed according to the classical technique - ligation of the anterior branch of the left coronary artery. The contractile activity was studied on the Langendorff System. The data were statistically processed using Student's t-test.

Results: A comparative analysis of the effect of MI on the initial values of contraction force in the studied groups revealed that in rats the contraction force decreased one day after MI and tended to increase 54 and 120 days after the simulation of MI.

Conclusions: Thus, it was shown that at different stages of the postinfarction period, multidirectional changes of the isolated rat heart myocardium contractions force are observed. The study was supported by Russian Science Foundation (grant No. 21-15-00121, https://rscf.ru/project/21-15-00121/)

56ASM-0102 | Effect of HCN channel blocker in the regulation of chronotropic effects in rats with limited motor activity

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Background: In the modern world, limitation of motor activity is an acute problem, because there are many reasons leading to this way of life. Hypokinesia causes atrophy of the musculoskeletal system, complicates the digestive, respiratory and cardiovascular systems. The involvement of HCN channels in the mechanism of heart rhythm acceleration has been shown. It is interesting to see the effect of blockade of If-currents and their role in the regulation of chronotropy of the heart against the background of increased heart rate (HR) in response to hypokinesia.

Materials and Methods: The experiments were conducted on two groups of rats: 1- control group, rats 7 weeks old; 2 - experimental group, rats with restriction of motor activity for 30 days. This effect was achieved by placing 3-week-old rats in penal cages under conditions of increasing hypokinesia. The effect of If blocker ZD7288 (10^{-9}M and 10^{-6}M) on chronotropic effects was studied using Langendorff PowerLab8/35 (ADInstruments, Australia).

Results: After the introduction into the perfused solution ZD7288 (10^{-9}M), a decrease in heart rate by 15% was observed in control rats ($p < 0.01$) and by 11% ($p < 0.05$) in the experimental group. The blocker If in concentrations 10^{-6}M decreased heart rate in the control group by 28% ($p < 0.01$) and by 17% in experimental group.

Conclusions: If-current blocker ZD7288 at all concentrations caused a decrease in heart rate in control rats and rats, with limitation of motor activity. However, more pronounced changes in heart rate were observed in the control group of rats and after application of the maximum concentration. It is possible that in rats with limited motor activity, against the background of an increase in heart rate, the density of HCN channels decreases compensatory, which leads to decrease their role in the regulation of heart rate. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0103 | Isolated rat heart after restriction of motor activity and recovery

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Background: Restriction of motor activity becomes a medical and social problem. Restriction of muscle activity leads to violations of all organ systems of the human body. Namely, in the cardiovascular system, prolonged restriction of motor activity leads to coronary vessels, the heart muscle weakening, decreasing of the heart energy potential and minute volume. The aim of our study was to study possible age-related changes in the parameters of the isolated rat heart after hypokinesia and subsequent recovery.

Materials and Methods: Restriction of motor activity was carried out by placing animals in pencil cases in conditions of increasing hypokinesia for 30 days. The recovery stage after hypokinesia for 14 days was carried out in order to study the mechanisms of adaptation of the animal to changes in the motor regime. The following parameters of the isolated heart were recorded - the pressure developed in the left ventricle (LVL), heart rate (HR) and coronary flow (CP) on the Langendorff PowerLab 8/35 unit (ADInstruments, Australia). Statistical processing was carried out in Excel, the reliability was determined using the Student's t-test.

Results: After hypokinesia, unidirectional changes in the parameters of the isolated heart were observed in 7-week-old and adult rats: a decrease in the parameters of the LVL, CP and an increase in heart rate. However,

during readaptation after hypokinesia, adult rats reacted with a tendency to restore LVL, CP and a complete restoration of heart rate values. The recovery period in rat pups led to a decrease in the parameters of LVL (29%) and CP (23%) below the control values and a decrease in heart rate parameters by 27% of heart rate ($p < 0.05$).

Conclusions: Thus, unlike adult animals, a recovery period of two weeks is insufficient for young developing rats. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0104 | Nitric oxide effect on rat myocardial contractility during mobility restriction

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Background: Nitric oxide (NO) is a signaling molecule involved in the regulation of myocardial contractility. The action of many drugs used in cardiology is based on the release of NO, but the vascular and cardiac effects are not fully understood. Research on the role of NO in the body during motor activity limitation is of interest. There is evidence that prolonged limitation of mobility causes significant changes in the contractile function of the heart.

Materials and Methods: Experiments were carried out on random-bred albino rats. Restrictions of motor activity were achieved by placing rats in a small box: the first two days, the time of inactivity was 1 hour, and then increased by 2 hours every 2 days. By day 25, the time spent by animals in the cage-cases reached 23 hours. We determined the response of ventricular myocardial contractile function to the action of SNP (SNP at a dose of 10-6M) and against the background of L-NAME at a dose of 10 mg/kg. The contractile activity of myocardium was examined in vitro in a PowerLab setup equipped with a MLT 050/D Force Transducer (ADInstruments). We calculated the response of contraction force in response to pharmacological agents as a percentage of the initial force (100%). Experiments were performed in accordance with the regulatory guidelines for the treatment of laboratory animals.

Results: Under the action of SNP there was an increase in ventricular myocardial striatal contraction force by 23% ($p < 0.05$). Against the background of the action of L-NAME ventricular myocardial stripe contractile force with the addition of SNP increased by 55% compared with the baseline ($p < 0.05$).

Conclusions: The positive effect of SNP is increased 2.5-fold in rats growing under mobility restriction against the background of non-selective NO synthase blockade. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0105 | In vivo ultrasonographic evaluation of skeletal muscle and cardiac function and structure in animal models of neuromuscular disorders: a new approach to improve preclinical translational research

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Background: Neuromuscular disorders induce structural and functional muscle changes relevant for diagnosis and disease progression. The absence, in many cases, of specific therapies makes it necessary to improve predictability of pre-clinical studies also regarding methodology. Ultrasonography is a useful method for assessing quantitative changes in human muscle such as muscle size and presence of fat or fibrous tissue infiltrations through echodensity measures. Today, it is possible to apply ultrasound in preclinical settings obtaining more predictive data to translate in patients. We recently set up an ultrasonographic technique for ultrasound acquisition suitable for rodent skeletal muscle and validated this new approach to assess disease progression and pharmacological efficacy.

Materials and Methods: Ultrasonography experiments were carried out using the Vevo2100 set up equipped with a probe working at 40 HMz (cardiac acquisitions) and a probe working at 21 MHz (diaphragm and hindlimb acquisitions).

Results: By ultrasound, we showed that the treatment with growth hormone secretagogues prevent the FDL muscle loss occurring in a rat model of cisplatin induced cachexia. Subsequently, we showed that the long-term treatment with taurine of mdx mice, a model of Duchenne Muscular Dystrophy, exerted a protective action improving the left ventricular function as demonstrated by the restoration of ejection fraction, shortening fraction, and stroke volume values.

In mdx mice, the morphological and functional properties of diaphragm muscle were investigated showing a significant decrease in diaphragm contractile amplitude and a significant increase in mean pixel echodensity as an index of fibrosis.

We extended our ultrasonographic investigations to gender-dependent muscle alteration in aging sarcopenia. We showed a severe atrophy occurring in sarcopenia which was more pronounced in male than in female rats.
Conclusions: Our studies corroborate the usefulness of ultrasound as a non-invasive tool to monitor muscle and cardiac alterations in several pathophysiological conditions introducing new markers of disease progression in translational research easily translatable into clinical settings.

56ASM-0212 | Cardiovascular & metabolic diseases modeling of ageing in human cardiomyocytes derived from induced pluripotent stem cells

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Background: It is estimated that, by 2030, the number of people over 60 years will grow by 56 percent. Ageing is a progressive functional degeneration of an organism and it is closely associated with the development of cardiovascular diseases. Age and disease models have been of great use for the understanding of the molecular mechanisms underlying cardiac ageing. In vitro models, however, have mainly focused on pathological pathways which are linked with impaired cardiac function, consisting in knockouts and knockdowns for assessment of protein-specific impact in cardiomyocyte function and pathology, but not on ageing itself. In the current study we investigate: (i) the impact of ageing in the differentiation of human iPSCs into left ventricle (LV) cardiomyocytes (CMs); (ii) the changes on the metabolism of these aged LV CMs; and (iii) the effect of drugs at the molecular, cellular and functional levels.
Materials and Methods: Through WNT signaling pathway modulation, human induced pluripotent stem cells (hiPSCs) derived from dermal fibroblasts of aged patients and a healthy control line were differentiated into left ventricular cardiomyocytes, in a 30-day differentiation protocol. On day 30, the CMs were characterized regarding mitochondrial respiration (resorting to a SeaHorse XF Mitro Stress Assay) and calcium handling (through fluorescence calcium imaging). The effect of a selection of bioactive compounds on these parameters was performed.
Results: Aged hiPSCs were successfully differentiated into LV CMs. The onset of beating (day 6) and cardiac differentiation efficiency (ca. 75% of CMs) were similar in both hiPSC and healthy control lines. Notably, aged-iPSC-CMs exhibited changes in mitochondrial respiration and calcium handling. Currently, a group of compounds is being screened as potential treatment strategies to revert the observed phenotype.
Conclusions: In this work, it was successfully generated a reliable in vitro model of human aged left ventricle cardiomyocytes, potentiating the study of the effects of ageing in human cardiomyocytes.

56ASM-0109 | NPY as a modulator of adrenergic influence on the electrical activity of rat cardiomyocytes

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Background: Neuropeptide Y (NPY) is the most abundant peptide in the heart. NPY has also been shown to be involved in the pathogenesis of cardiovascular diseases, including hypertension, myocardial hypertrophy, atherosclerosis, ischemia, infarction, arrhythmias, and heart failure. The aim of this studing is to study the modulating effect of the agonist Y1,5-receptors [Leu31, Pro34]NPY on the electrical parameters of the right atrium with preserved sinus node and spontaneous activity in 21-day-old animals.
Materials and Methods: The study was carried out on laboratory rats of 21-days old ($n = 20$). We studied the electrical activity of cardiomyocytes using an intracellular microelectrode lead. We prepare the right atrial myocardium with a preserved sinus node and spontaneous activity. Statistical significance was carried out using paired Student's t- test. To study the possible influence of [Leu31, Pro34]NPY on the effects of isoproterenol, we added a selective agonist against the background of isoproterenol.
Results: The β1,2-adrenoreceptor agonist isoproterenol 10mol/L increases the frequency of action potential (AP) generation by 35% ($p < 0.01$), but does not change the AP amplitude. The combined administration of isoproterenol and an NPY1.5 agonist reduces the frequency of AP generation from 35% to 13% ($p < 0.05$), but does not change the AP amplitude. It should be noted that with the combined administration of agonists, the effect of isoproterenol on the duration of AP decreased. Isoproterenol reduces APD20, APD50 and APD90 by 19%; 24%; 17%, respectively

($p < 0.01$; $n = 10$). Combined application of agonists reduces the duration of the repolarization phase only due to APD50 ($p < 0.05$; $n = 10$).

Conclusions: Thus, activation of NPY receptors inhibits adrenergic influences on the frequency of AP generation of the rats atrial myocardium. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)

56ASM-0110 | Negative ultraslow potentials in the rat cerebral cortex during terminal ischemia

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Background: Negative ultraslow potentials (NUPs) attaining giant values of up to -150 mV were recently discovered using Pt/Ir electrodes in the cerebral cortex of patients with focal and global brain ischemia. However, generative mechanisms of NUPs remain poorly understood.

Materials and Methods: Here, we explored negative ultraslow potentials using direct-coupled recordings of the intracortical potential with 16-channel Ir probes and AgCl/glass-pipette electrodes in the rat barrel cortex ($n = 12$ rats) during global terminal ischemia evoked by lethal isoflurane inhalation. Reference AgCl-electrode was placed in cerebellum.

Results: Isoflurane-induced respiratory and cardiac arrest caused immediate suppression of the ongoing electrographic activity followed by complex slow shifts of the extracellular field potential. First, a 2-3 min long positive voltage shift was observed attaining on average +23 mV and +5.5 mV at Ir- and AgCl-electrodes, respectively. Spreading depolarization (SD) marked further positive to negative switch in the field potential, and heralded NUP. NUP developed through two consecutive phases of slow and infraslow depolarization. The slow phase lasted for approximately 15 min attaining on average -55 mV at Ir- and -8 mV at AgCl-electrodes. Further ultraslow process continued for the next 3-4 hours failing to attain steady-state levels by the end of recordings. Polarity and magnitude of the voltage change during ultraslow phase differed between electrodes, attaining nearly -150 mV at Ir-electrodes but positive and variable voltage shifts ranging from +8 to +85 mV at AgCl electrodes.

Conclusions: We show that NUP is reproducible in the global and terminal rat brain ischemia model. NUPs' features, particularly during the late ultraslow phase, strongly depend on the electrode material suggesting a contribution of non-biological processes to the signal.

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56ASM-0113 | Parameters of the action potential of the atrial myocardium during the blockade of NPY1-receptors in ontogenesis

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Background: Neuropeptide Y (NPY) is a highly conserved peptide that acts through a family of G protein-coupled receptors. The action of NPY in the myocardium is realized with the various types of receptors, which have both pre- and postsynaptic location. NPY can regulate blood pressure, psychomotor function, anxiety, food intake, and endocrine secretions. BIBP 3226 is a selective non-peptide antagonist of NPY_1-receptors. The aim of this study is to study the effect of the selective antagonist of NPY_1- receptor BIBP 3226 on the electrical activity of the right atrial preparations.

Materials and Methods: The study was carried out on laboratory rats at 21- and 100- days old ($n = 18$). Animals anesthetized with urethane, their hearts took off and placed in an oxygenated Tyrode solution. The preparation of the right atrium with preserved sinus node and spontaneous activity was made. We placed the preparation in a chamber where a thermostatic solution (37 ± 1°C) was supplied. We studied the electrical activity of cardiomyocytes using an intracellular microelectrode. Statistical significance was carried out using paired Student's t- test.

Results: According to the literature, BIBP 3226 at a concentration of 10^{-6}mol/L completely blocks NPY1 receptors. The application of the blocker in 21-day-old animals reduces the repolarization duration of action potential (AP) in cardiomyocytes at the levels APD20, APD50 and APD90 by 9%; 15%; 9%, respectively ($p < 0.05$; $n = 9$). In 100-day-old animals, APD20, APD 50 decreased by 29%; 28%, ($p < 0.05$; $n = 9$). Membrane potential, frequency of spontaneous activity and amplitude AP do not change significantly in 21- and 100-day-old animals.

Conclusions: Thus, blockade of NPY1 receptors leads to a decrease in the duration of AP in 21- and 100-day-old animals, which proves the participation of this type of receptors in the formation of the electrical activity of right atrium cardiomyocytes. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0115 | The duration of emergency department staying predicts late SARS-CoV-2 positivity in italian internal medicine wards

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Background: During the first wave of SARS-CoV-2 pandemic, preventing the spreading of the infection was particularly difficult since few data were available to recognize high-risk patients. Consequently, many cases remained undiagnosed. The aim of our study was to determine the clinical predictors of late nasopharyngeal swab positivity after admission in a COVID-free internal medicine ward in order to stratify patients and preventing clusters of disease.
Materials and Methods: Patients admitted to a SARS-CoV-2-free internal medicine ward at the IRRCS Ospedale Policlinico San Martino in Genoa between February 2020 and May 2020 were included in this retrospective study. The overall cohort consisted of 478 patients that were assessed via clinical and biochemical evaluations. Cases of late positivity were defined as patients testing positive for SARS-CoV-2 in internal medicine wards not SARS-CoV-2-dedicated after previous negative molecular test performed in emergency department (ED). The primary endpoint was to identify predictors of late positivity to SARS-CoV-2 infection. Secondary outcomes included overall and SARS-CoV-2-related mortality.
Results: Patients were prevalently elderly, 48.3% were male. The median days of incubation were six. A longer staying at the ED was associated with higher risk of late nasal swab positivity even for small differences. Fever, dyspnea, and duration of ED hospitalization were independently associated with late SARS-CoV-2 positivity at the adjusted logistic regression model. We internally validated the model and the ROC curve showed adequate specificity and sensibility. Furthermore, at Cox regression analysis, the duration of ED hospitalization emerged as an independent predictor of in-hospital mortality. The overall mortality was 12.6% ($n = 60$).
Conclusions: Here we report that the duration of stay at the ED before the admission to ordinary wards represents a potential risk factor for late in-hospital positivity to SARS-CoV-2 diagnostic tests. Despite the implementation of prevention protocols, the high variability of virus incubation time might determine a failure of preventive measures.

56ASM-0132 | Uric acid induces vascular smooth muscle cell migration through angiotensin II and proteasome

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Background: Growing evidence indicates that high levels of uric acid (UA) are a risk factor for the development of cardio-renal disease because of the involvement in vascular inflammation and remodeling. Vascular smooth muscle cells (VSMCs) are highly plastic cells able to move from a contractile to a secretory state. Whether UA is involved in this VSMC phenotypic transition is not yet known.
Materials and Methods: MOVAS, a mouse VSMC cell line, was exposed to 6, 9 and 12 mg/dL of UA or control for 24-48 hrs. Proliferation and migration were then tested alongside with the effect of atrogin-1 and its suppression by MG132. The involvement of angiotensin II (A2) was finally tested through AT1 Receptor (AT1R) mRNA quantification and its blocking with losartan (L) or valsartan (V) (both at 10 μmol).
Results: UA had no effects on cell viability but small increase of cell proliferation at 24h (+11-15%). Rather, UA promoted the migratory rate at 24h and 48h ($p < 0.001$), confirmed by phalloidin staining. UA also significantly increased smoothelin B, αSMA and SM 22α levels at 24 h (+20/50%), with a subsequent drop at 48h. VSMCs also increase their area (+ 30%) in response to UA stimulation, in a time- and concentration-independent manner. Atrogin-1 was 2-fold up-regulated at 48h after UA exposure ($p = 0.04$) with an inverse correlation between its protein and αSMA expression. MG132 instead blunted the migration and F-actin re-arrangement. Similarly, L and V blocked VSMC migration ($p < 0.001$ vs UA) and finally

inhibited UA-induced increase in cells area ($p < 0.001$ vs UA).

Conclusions: Our findings indicate UA as key player in vascular remodeling through detrimental effects on VSMC proliferation, cell area, migration and cytoskeleton. A2 and proteasome inhibitors may rescue VSMC from such a detrimental phenotypic with protective effects on cardio-renal disease induced by UA.

56ASM-0145 | Risk assessment of the incidence of cardiovascular diseases on the basis of evolutionary model

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Background: We carried out the testing of the risk assessment for the population health in the city of Kazan on exposure to chemicals coming with the atmospheric air (PM10, CO, NO2, SO2) on the basis of evolutionary model.

Materials and Methods: The coefficients taking into account the risk evolution due to natural causes (αi) are determined on the basis of the morbidity and mortality background indices for the disease classes reflecting functional disorders of the critical organs and systems. The health data, which are typical for the most favorable regions as far as environmental pollution, are chosen as the background levels [MR 2.1.10.0062 – 12].

Results: The cardiovascular system were found to be the critical organs for exposure to mentioned major pollutants. The results show that the additional risk (ΔR) of the functional disorders accumulation under chosen scenario at the level of reference concentrations of chemical substances in the atmospheric air is formed earlier for the cardiovascular system than for the respiratory system, at the level of 0.6 – by the age of 40 and 82 years, and at the level of 1.0 - by the age of 51 and 93 years, respectively. The risk structure, changes depending on the age and the factors' exposure duration. It was established based on the indices of additional risk and presented risk (R) index that the unacceptable (moderate) health risk ($R = 0{,}05$-$0{,}35$) was formed by the age of 27 years, and after the age of 40 years, it could be classified as high ($R = 0{,}35$-$0{,}6$).

Conclusions: The proportion of additional risk for the cardio-vascular system (ΔR CVS) of the aggregate risk value at the age of 10 years makes 12.5% and 77. 5 % % at the age of 70 years. The data obtained adequately reflect the current epidemiological and ecological situation in the city territory in recent years

56ASM-0157 | Does early vascular aging predict future acute coronary syndrome cases?

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Background: Acute coronary syndrome (ACS) is associated with high number of pre-hospital and hospital mortality as well as morbidly. Modern cardiology are in need of defining early prognostic markers in patients presenting ACS. We hypothesized if early vascular aging may be associated with the coronary artery disease (CAD) course and severity, and is a predictor for ACS.

Materials and Methods: 120 patients with stable CAD validated on coronary angiography were enrolled in the study (mean age was 58.45 ± 14.60 years, male = 59%). Early vascular aging was defined using baseline tonometry and pulse wave velocity. Acute coronary syndrome was diagnosed according to the latest international guidelines. Multivariable logistic analysis was used to assess possible predictive value of early vascular aging. All models were adjusted with variables and risk factors and β value was evaluated to compare sizes.

Results: Mean calendar age was 58.45 ± 14.60 years and mean vascular age 65.25 ± 15.80 years. Among 46 percent of patients with early vascular aging, men were 62 %. Mean follow-up time was 4.6 ± 2.5 years. Linear regression analysis revealed that advanced vascular arterial aging (β: 0.182, $P < 0.05$) was associated with ACS whilst calendar age (β: 0.045, $P > 0.05$) was not. Older vascular aging was associated with 2.2 time higher increase of ACS cases whereas older calendar age was only 1.2-fold increase. When we analyzed mortality from ACS, advanced vascular aging was significantly associated with it ($P < 0.05$) and was independent predictor for it (β: 0.247,

$P < 0.05$). Even though calendar age was slightly associated with mortality it was not predictor for it (β: 0.013, $P > 0.05$).

Conclusions: Early vascular aging is an independent risk factor of acute coronary syndrome and seems to be superior to calendar age in the course of disease. Further studies are needed with large amount of patients to clarify.

56ASM-0158 | Clopidogrel efficacy after percutaneous coronary interventions considering with cyp2c19 genetic polymorphisms in patients with coronary heart disease

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Background: Latest data suggest that efficacy of antiplatelet therapy with clopidogrel mainly dependent on genetic variants of CYP2C19 genes. Aim of the study was to estimate the impact of CYP2C19 genetic polymorphisms on the antiplatelet response with clopidogrel and their possible relationship between mean platelet volume in patients with coronary heart disease (CHD) after percutaneous coronary interventions (PCI).

Materials and Methods: 110 patients (aged 42-75 years; mean age 55.40 ± 14.2; male $n = 52$) with CHD who were underwent percutaneous coronary interventions followed by stenting with DES were enrolled in the study. Blood samples for platelet function testing were collected at baseline and after the twelve hours with six hundred mg loading dose of clopidogrel. Platelet aggregation was performed in a 2-channel aggregometer and assessed by inhibition of platelet aggregation. Genetic polymorphisms of the CYP2C19 were performed using polymerase chain reaction. Platelet count and mean platelet volume (MPV) were assessed.

Results: Among 110 patients, 48% of patients had CYP2C19*1, 21 % CYP2C19*2, 11% CYP2C19*3 and 20% CYP2C19*17 genetic polymorphisms. Only 84.0 % of patients had a response to clopidogrel. Most of the non-responders were subjects with CYP2C19*2 and CYP2C19*3 genotypes. Inhibition of platelet aggregation significantly increased in 12 hours after loading dose in CYP2C19*17 genotype with both 5 mmol/L, and 20 mmol/L ADP ($P < 0.05$), and normally increased after 12 hours with loading dose in CYP2C19*1 genotyping subjects ($P < 0.05$) whilst IPA was not changed significantly in subjects with CYP2C19*2 and CYP2C19*3 genetic polymorphisms with 5 mmol/L and 20 mmoll/L ADP ($P > 0.05$). Platelet count did distinguish in all genetic variants. Mean platelet volume was larger in non-responders than responders ($P < 0.05$).

Conclusions: Among genetic variants, carriers of the CYP2C19*2, CYP2C19*3 single nucleotide polymorphisms are predictor for clopidogrel resistance whereas CYP2C19*17 polymorphisms are strong responders in our subjects. Platelet count didn't differ in all variants while MPV tended to be smaller in responders.

56ASM-0159 | Plasma B-type natriuretic peptide in patients with coronary artery disease and metabolic syndrome

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Background: Number of studies demonstrated that plasma b-type natriuretic peptide (BNP) level was associated with future adverse cardiac events. Objectives of the study was to assess the possible prognostic value of BNP in patients with coronary artery disease (CAD) and metabolic syndrome (MetS).

Materials and Methods: One hundred and sixty patients with CAD with MetS were enrolled in the study from 2016 to 2018 years (aged 36-72 years, mean age – 56.4 ± 12.0 years, male-46%). Patients were divided into two groups according to the plasma concentration of BNP. Group I – 97 patients with increased BNP (> 30 pg/mL) and Group to – 63 patients not increased BNP (< 30pg/mL). Mean follow-up period was 3.2 years. Major adverse cardiovascular events (MACE) were defined as composite of revascularization due to unstable angina or acute coronary syndrome, myocardial infarction, stroke, death).

Results: During the mean follow-up period there were increased number of adverse cardiac events in Group I after

adjustment (2.62, 1.65-3.25, CI 95%, $P < 0.05$) than Group II (2.10, 1.40-3.15, CI 95%, $P < 0.05$). During the follow-up patients who had repeatedly high level of BNP (more than 30 pg/mL) had higher rate of MACE (2.96, 1.75-4.55, CI 95%, $P < 0.05$) than only had higher level of BNP at baseline and during follow-up checkups had lower BNP (2.15, 1.35-3.24, CI 95%, $P < 0.05$). Among MetS components abdominal obesity (1.8, 1.1-2.4, CI 95%, $P < 0.05$), hypertension (1.65, 1.08-2.20, CI 95%, $P < 0.05$) and dyslipidemia (1.42, 1.05-1.80, CI 95%, $P < 0.05$) were predictors for the MACE.

Conclusions: Increased plasma levels of BNP were associated with major adverse cardiovascular outcomes in patients with CAD and MetS.

56ASM-0161 | Influence of diet with low glycemic index on anthropometric parameters in patients with atherosclerotic coronary artery disease

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Background: Diet with low glycemic index (DLGI) has been demonstrated favorable effect on several biochemical indexes. Purpose of the study was to evaluate the diet with low glycemic index on anthropometric parameters such as on body mass index (BMI), waist circumference (WC) and hip circumference (HC), waist to hip ratio (WHR) in patients with atherosclerotic coronary artery disease (ACAD).

Materials and Methods: 160 patients with confirmed ACAD, aged 38-76 years old (mean age 58.2 ± 12.0 years; male = 48%) entered as three months dietary intervention either with DLGI ($n = 80$) or routine diet (RD, $n = 80$) together with standard therapy from 2016 to 2019. BMI, WC, HC, WHR were checked at baseline and in three months of the intervention. All statistical analysis were performed by STATA software.

Results: DLGI showed favorable effect on BMI (from 29.8 ± 8.12 to 91 kg/m2 in Group 1 vs. from 30.2 ± 9.30 to 28.8 ± 8.7 kg/m2 in Group 2, $P < 0.05$), WC (from 100.6 ± 18.5 cm to 91.6 ± 16.8 cm vs. from 98.9 ± 19.2 cm to 95.6 ± 18.1 cm, $p < 0.05$), HC (from 95.8 ± 20.2 cm to 87.4 ± 18.4 cm vs. from 97.0 ± 19.8 cm to 96.2 ± 19.5 cm) and WHR (from 1.1 ± 0.9 cm to 0.9 ± 0.8 cm vs. from 1.0 ± 0.9 cm to 0.98 ± 0.78 cm) than routine diet. Even though, BMI, WC, HC and WHR decreased in both groups from baseline, there were only statistically significant changes in Group 1 compared to Group 2. When we separately analyzed gender differences men tended to be more likely to be influenced by diet with low glycemic index ($P > 0.05$) than women in terms of WC, HC and WHR however there were not observed significantly changes in BMI by gender.

Conclusions: DLGI showed superiority to reduce BMI, WC, HC and WHR patients with confirmed ACAD. Men had a tendency to be affected by WC, HC and WHR in DLGI intervention. Further studies are required with large amount of patients to clarify.

56ASM-0164 | Specificity of the development of acute coronary syndrome/acute myocardial infarction in men of various ages in one of the districts of tashkent

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Background: To study specific features of the development and progressing of the acute coronary syndrome (ACS) and acute myocardial infarction (AMI) in men in different age groups in one of the districts of Tashkent.

Materials and Methods: The registry included data about men – (464), in the framework of a cohort prospective investigation "Registry of acute coronary syndrome (ACS) and acute myocardial infarction(AMI) in one of the districts of Tashkent". There were used population-prophylactic, statistic, and mathematic methods of investigation.

Results: Hospital admission were 262 patients (56.4%), 202 patients (43.6%) died at the prehospital stage ($\chi 2 = 2.380$ $p = 0.018$): with "determined" AMI – 158 (78.2%) and "possible" AMI – 44(21.8%) ($\chi 23.219$ $p = 0.002$). Analysis by 5-year age groups showed that frequency of ACS/AMI increased with age, and at the age of 60-64 years there has been the highest prevalence rate (24.2%) ($p = 0.001$). Analysis of the patients hospitalization's ratio (analysis included 262 hospitalized patients) from the moment of beginning of the pain attack showed that only 32 (12.2%) men admitted during first 2 hours, of them only 5(1.9%) patients were presented into the hospital during "the golden first hour" when it was possible to achieve maximum effect of antithrombotic therapy. During the first 2

hours from the moment of the pain appearance the pain in the chest of the age group of 40-49 years the frequency of referral into the medical services accounted for 14.3%. The men frequently addressed for medical aid during the first 3 hours in the age group 60-69 years (16.9%). The special attention was given to the very high percent of patients admitted very late, more than on a day after appearance of pains (38.2%).

Conclusions: Prevalence and morbidity from ACS/AMI among men are high in our cohort. The most distribution rate of ACS/AMI was found at the age group 60-64 years old.

56ASM-0165 | Features of platelet induces in patients with atherosclerotic coronary artery disease

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Background: Emerging data show that platelet induces such as mean platelet volume (MPW) and platelet distribution width (PDW) might be associated with the severity of coronary artery disease. We aimed to evaluate the possible links the platelet induces with the severity of coronary artery disease.

Materials and Methods: 120 patients with CAD were enrolled in this prospective study (Aged 45-72 years, mean age 57.65 ± 14.80 years, male = 46%). Patients were divided into two groups by 60 due to the severity of CAD validated in coronary angiogram. Group I consisted of one or two vessel CAD patients whilst Group II included multi vessel CAD patients. All anthropometric, laboratory and instrumental data were obtained and all statistical analysis were performed by STATA software.

Results: Platelet volume (PV) and and plateletcrit (PCT) was similar in both groups (192 ± 24 10^9/L vs. 187 ± 26 10^9/L for PV and 0.23 ± 0.06 % vs. 0.24 ± 0.07 % for PCT), however mean platelet volume was higher in Group II than Group II (11.12 ± 2.4 fl vs. 9.85 ± 2.2 fl, $P < 0.05$). Concerning platelet distribution width, this index was higher in Group II than Group I, however these changes were not statistically significant (17.5 ± 3.2 % vs. 16.9 ± 2.9 %, $P > 0.05$). Among biochemical parameters high LDL-C level (0.61, CI 95%, $P < 0.05$), high glycated hemoglobin level (0.57, CI 95%, $P < 0.05$) and among anthropometric indexes high waist circumference (0.56, CI 95%, $P < 0.05$) positively associated with high MPV in patients with CAD.

Conclusions: High mean platelet volume might be associated with severe coronary artery disease whereas platelet distribution width was not associated with CAD.

56ASM-0171 | The effect of long-term physical exercise in older adults with multimorbidity: the impact on systemic inflammation

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Background: Aging is associated with an increased risk of cardiometabolic disorders, associated with chronic low-grade inflammation. Physical exercise promotes an anti-inflammatory status improving the plasma concentration of anti-inflammatory markers, which positively impact on the chronic inflammation reduction in older adults. The aim of this study was to test the effect of an exercise intervention on pro-and anti-inflammatory markers in older adults with multimorbidity.

Materials and Methods: 65 older women (82 ± 6.3 years old) participated in the study and were allocated in two groups: multicomponent exercise training (MET; $n = 31$) and non-exercising control group (CG; $n = 34$). The MET group performed progressive exercise intervention with 2-3 sessions/weekly, for 28 weeks. The CG did not alter their usual routine. Plasma levels of IL-10, TNF-α, and TNF/IL-10 ratio were analyzed before and after the 28 weeks exercise program.

Results: Coincident higher IL-10 levels and lower increases in TNF-α were observed ($p > 0.05$) in the MET group compared to CG. A lower TNF-α/IL-10 ratio was

observed only in the MET group while GC did not present any changes in the inflammatory markers or in their ratio, ($p > 0.05$).

Conclusions: The evidence regarding the use of moderate long-term exercise as a tool for promoting a better balance between pro- and anti-inflammatory status, in association with the reduction of the inflammatory index in the MET group seem to confirm the beneficial effects of exercise in reducing systemic low-grade chronic inflammation, even in older women with several co-morbidities.

56ASM-0189 | NOACs and warfarin prescription trends in portuguese outpatients

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Background: Cardiovascular diseases are the leading cause of mortality and disability worldwide. Older adults are more likely than the remaining population to suffer from these diseases in aging countries such as Portugal, thus causing an economical burden on National Health services. Currently, therapy with oral anticoagulants plays an important role in the prevention and treatment of thromboembolic events. This study aims to analyze the prescription of novel oral anticoagulants (NOACs) over time and compare these trends of prescription with the consumption of warfarin in Portuguese older adults.

Materials and Methods: A retrospective ecological study was performed between 1 January 2019 to 31 December 2021 in a national public database for the following prescribing oral NOACs (Apixaban, Dabigatran, Edoxaban, and Rivaroxaban) and Warfarin for older adults in mainland Portugal. Data were analyzed in terms of DDD and prescribed packages frequency, DDD per 1000 inhabitants (DID) and DID change rate (%).

Results: The most prescribed NOACs over the 3 years was Rivaroxaban with about 2.576.646 prescribed packages (31.62% of the total prescriptions), with a positive variation of 45%. In turn, the least prescribed NOAC was Dabigatran with 1.038.040 prescribed packages corresponding to 12.74% of the total prescriptions, with a positive variation of only 13%. The prescription of Warfarin decreases by 26.0% over the period in the study.

Conclusions: The use of NOACs has considerably changed the perspective of therapy compared to treatment with Warfarin since over the years there has been a progression in the increase in the prescription of NOACs in clinical practice.

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56ASM-0192 | Combined-exercise training improves PON-1 activity and systemic inflammatory status in older adults

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Background: Increased risk of cardiometabolic disorders in older adults is closely associated with the reduction of HDL-bound paraoxonase-1 (PON-1) activity and elevated systemic levels of pro-inflammatory cytokines. Although combined-exercise training (CET), which includes aerobic and resistance exercises, can ameliorate the inflammatory status and PON-1 activity, the benefit of CET in older adults is unclear. Therefore, we aimed to investigate whether the long-standing regular practice of CET could

improve the systemic inflammatory status and PON-1 activity in older adults.

Materials and Methods: Fifty-three older adults (age 72.5 ± 5.9 years) were separated into the CET group ($n = 36$) and the non-exercising group (CTRL; $n = 17$). The CET group performed the exercise protocol for, at least, 24 months. We assessed the HDL-linked PON-1 activity and systemic levels of pro- (IL-6 and TNF-a) and anti-inflammatory (IL-10) cytokines.

Results: Higher PON-1 activity ($p < 0.05$) and systemic IL-10 levels ($p < 0.05$), besides lower TNF-a/IL-10 ratio ($p < 0.01$), were found in the CET group compared to CTRL. Moreover, positive correlations between cytokines and a negative correlation between PON-1 activity and TNF-a levels were identified.

Conclusions: Long-standing regular practice of CET was able to improve the PON-1 activity and systemic inflammatory status in older adults.

56ASM-0003 | Differential expression of ATP-sensitive potassium channel subunits in bypass grafts from patients with and without type-2 diabetes mellitus

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Background: The mechanism of vasodilatory action of pinacidil includes interaction with smooth muscle ATP-sensitive potassium (K_{ATP}) channels. Previous studies have shown decreased vasodilation of blood vessels in different cardiovascular and metabolic conditions. Thus, the aim of our study was to detect the differences in the expression of K_{ATP} channel subunits in bypass grafts, human saphenous vein (HSV) and human internal mammary artery (HIMA), obtained from patients with and without T2DM.

Materials and Methods: Using organ bath system vasodilation induced by pinacidil was tested on segments of HSV and HIMA obtained from patients with and without T2DM who undergoing coronary bypass surgery. The expression of K_{ATP} subunits (Kir6.1, Kir6.2 and SUR2B) were detected by western blot and immunohistochemistry using segments of HSV and HIMA.

Results: Pinacidil induced vasodilation of bypass grafts, but its potential to produce vasodilation is different in segments obtained from patients with DM2T compared to segments obtained from patients without DM2T. All three types of K_{ATP} subunits are expressed on bypass grafts from patients with and without T2DM. While there is no difference in the expression of SUR2B subunit, the expression of the Kir6.1 and Kir6.2 subunits are lower in HIMA obtained from patients with T2DM ($P < 0.05$). Otherwise, was observed in HSV. The expression of SUR2B subunit is lower in HSV obtained from patients with T2DM ($P < 0.05$).

Conclusions: The K_{ATP} channels are expressed in the vascular smooth muscle of bypass grafts, but they are partly involved in the dilatation of bypass grafts induced by pinacidil. It seems, that pinacidil has additional mechanism(s) of action. Also, this could implicate that the presence of diabetes decreasing the level of the expression of different subunits of K_{ATP} channels, and makes the differences in produced vasodilation by pinacidil.

56ASM-0212 | Modeling of ageing in human cardiomyocytes derived from induced pluripotent stem cells

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Background: It is estimated that, by 2030, the number of people over 60 years will grow by 56 percent. Ageing is a progressive functional degeneration of an organism and it is closely associated with the development of cardiovascular diseases.

Age and disease models have been of great use for the understanding of the molecular mechanisms underlying cardiac ageing. *In vitro* models, however, have mainly focused on pathological pathways which are linked with impaired cardiac function, consisting in knockouts and knockdowns for assessment of protein-specific impact

in cardiomyocyte function and pathology, but not on ageing itself.

In the current study we investigate: (i) the impact of ageing in the differentiation of human iPSCs into left ventricle (LV) cardiomyocytes (CMs); (ii) the changes on the metabolism of these aged LV CMs; and (iii) the effect of drugs at the molecular, cellular and functional levels.

Materials and Methods: Through WNT signaling pathway modulation, human induced pluripotent stem cells (hiPSCs) derived from dermal fibroblasts of aged patients and a healthy control line were differentiated into left ventricular cardiomyocytes, in a 30-day differentiation protocol. On day 30, the CMs were characterized regarding mitochondrial respiration (resorting to a SeaHorse XF Mitro Stress Assay) and calcium handling (through fluorescence calcium imaging). The effect of a selection of bioactive compounds on these parameters was performed.

Results: Aged hiPSCs were successfully differentiated into LV CMs. The onset of beating (day 6) and cardiac differentiation efficiency (ca. 75% of CMs) were similar in both hiPSC and healthy control lines. Notably, aged-iPSC-CMs exhibited changes in mitochondrial respiration and calcium handling. Currently, a group of compounds is being screened as potential treatment strategies to revert the observed phenotype.

Conclusions: In this work, it was successfully generated a reliable *in vitro* model of human aged left ventricle cardiomyocytes, potentiating the study of the effects of ageing in human cardiomyocytes.

56ASM-0213 | Uses of predictive immunological biomarkers for the diagnosis and control of the evolution of different clinical forms of cardiovascular disease

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Background: Coronary heart disease is a major health problem worldwide. One of the current challenges related to its prevention is that of early diagnosis. Literature data suggest the use of immunological biomarkers for the diagnosis and course of disease.

Materials and Methods: This study focuses on the search for predictive immunological biomarkers for the diagnosis and control of the evolution of different clinical forms of cardiovascular diseases. Anti-DNA antibodies were searched in the blood serum of 57 patients with cardiovascular disease of various forms and 27 relatively healthy donors who gave their consent. The determination of the level of anti-DNA antibodies was carried out by enzyme immunoassay (ELISA). The catalytic activity of the anti-DNA antibody was estimated by the transformation of the super coiled plasmid DNA pBR322 into circular and linear form by the agarose gel electrophoresis method.

Results: The results showed a significant increase in the levels of anti-nDNA Ac and anti-nDNA Ac between groups of patients with cardiovascular disease. A high anti-adna Ac level was observed in 70.70% of patients with cardiovascular diseases. The anti-DNA antibodies found in the serum of the different groups exhibit catalytic activities and are able to transform the super-coiled DNA into linear and circular DNA as a function of incubation time.

Conclusions: From these data, it appears that anti-DNA autoantibodies play a preponderant role in the pathogenesis of cardiovascular diseases and could be used as a biomarker in the diagnosis of this disease.

56ASM-0243 | Paroxysmal supra-ventricular tachycardia and massive pulmonary embolism in SARS-CoV-2

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Background: Pulmonary embolism (PE) has been described as a common and relevant complication of Sars-CoV-2 infection. In addition to the damage directly caused by the virus, other factors are also involved in this disease, such as immune system, hypoxia, bed rest, inflammation, age, smoking habit, and endothelial dysfunction.

Materials and Methods: Here, we describe the case of a 68-years-old female patient hospitalized for severe respiratory failure and SARS-CoV-2 infection. She had performed a chest CT scan in the emergency room, which documented the presence of massive and diffuse embolism in both branches of the pulmonary artery, with greater involvement on the left. in addition, CT lung scan showed clear signs of bilateral interstitial pneumonia, more extensive on the right lung.

Results: After three days, the patient appeared sedated and showed severe hypotension (80/60 mm/Hg). An

electrocardiogram documented the presence of paroxysmal supra-ventricular tachycardia (AVNRT) at a rate of 160 /min. A rapid infusion of amiodarone began (2.5 mg/kg in 250 of glucose) at the same time, with rapid successful, while a carotid sinus massage was immediately performed. The coexistence of an extensive embolism with the sudden increase in heart frequency had suddenly compromised cardiac hemodynamic, with severe hypotension. The pharmacological interruption of the arrhythmia resulted in a prompt recovery of blood pressure values.

Conclusions: After few days, the patient recovered also from SARS-CoV-2 infection: indeed, a big improvement of alveolar changes, together with a reduction of symptoms, were rapidly observed. In conclusion, the correct and timely management of arrhythmia and of cardiac complications might have earlier improved the outcome of this devastating disease.

56ASM-0266 | Detection of ST-elevation on the electrocardiogram recorded using a precordial dry 4-electrode arrangement

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Background: Cardiovascular diseases are the leading cause of mortality for an estimated 17.9 million patients annually, with the main contributor being ischemic heart disease. To diagnose various cardiac diseases, the heart can be monitored using a variety of electrocardiogram (ECG) devices. ECG devices have moved from clinical 12-lead to patches and wearables with 1 to 3 leads. Most devices focus on rhythm disorders. We developed the miniECG, a portable, smartphone-sized, multi-lead precordial ECG recording device.

The aim of our study was to investigate the ability of the miniECG to detect ST-deviations in 350 patients presenting with chest pain in two large Dutch hospitals.

Materials and Methods: Between May and December 2021, patients presenting with acute chest pain at the emergency department of the University Medical Center Utrecht or Meander Medical Center were included in the study. 12-lead ECGs were obtained in either the ambulance or hospital, miniECG recordings were obtained on arrival in the hospital. For comparison between 12-lead ECG and miniECG recordings, only patients with confirmed ST-elevation myocardial infarction (STEMI) and miniECG recordings of sufficient quality were included.

Results: A total of 268 patients were included, 40 demonstrated ST-elevation on their 12-lead ambulance ECG. In 33 of these patients the ST-elevation was also present on the miniECG recording (11/13 anterior, 16/21 inferior, 4/4 lateral and 2/2 other). For 4/7 of the other patients, ST-elevation had resolved on the follow up 12-lead clinical ECG and for 3/7 of the patients no additional 12-lead recording was performed at the hospital.

Conclusions: The miniECG records high-quality multi-lead ECGs. Preliminary analysis of clinical study data shows that the miniECG can accurately detect ischemic ST-elevation in patients with inferior, lateral, and anterior myocardial infarction. Further research is required to demonstrate non-inferiority of the miniECG to the standard 12-lead ECG in the detection of other cardiac (ab) normalities.

SYMPOSIUM 4: BIOINFORMATICS AND COMPUTATIONAL BIOLOGY FOR BIOMEDICINE

56ASM-0280 S4 IS | An embedding space for SARS-CoV-2 epitope-based vaccines

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Epitopes are relatively small peptide chains which play an important role in the immune response. They are identified by T cells and B cells which participate in the immune response in humans. Epitope-based vaccines synthesize epitopes that when inoculated stimulate the natural response to a pathogen. This type of vaccines have been proposed for pathogens such as influenza, tuberculosis, dengue, and more recently SARS-CoV-2.

In this talk we address the problem of learning an embedding representation of epitopes useful for the design of epitope vaccines. Different criteria should be taken into account in the design of an epitope vaccine, including the biding affinity of the epitopes to one or more major histocompatibility complex (MHC) alleles, the extent to which they cover haplotype distribution of the target population, etc. The goal of the epitope embedding design is capturing in the representation specific immunogenic characteristics of the epitopes in relation to the different MHC alleles. Starting from an original set of peptides (peptide vocabulary), e.g., those extracted from the genome of a pathogen, we propose methods to generate artificial sequences of

such peptides. The sequence generation method is used to create large datasets of sequences (vaccine corpora) which are assumed to exhibit some latent semantics related to the way epitopes interact among them and with alleles to provide an immunogenic effect. We use the corpora to create neural epitope embeddings learned in an unsupervised way. We them explore the space of embeddings and discuss how to use them to define intrinsic and extrinsic tasks related to vaccine design.
Using a large set of SARS-CoV-2 T cell epitope candidates, we show how to address the vaccine design problem in the epitope embedding space, and provide evidence that such embeddings can be used for solving downstream tasks related to epitope-based vaccine design.

56ASM-0308 S4 IS | Vertex model of the pseudo-stratified neural tube epithelium

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Background: The mechanisms that control the spatial organisation of cells in a developing tissue involve molecular, cellular and mechanical processes. Cells proliferate and the tissue grows as the patterns of gene expression that control cell fate are established. This means that cell adhesion, movement, division and differentiation contribute to the elaboration of developmental pattern and the arrangement of cells within a tissue. This is the case in the vertebrate neural tube where neurons differentiate in a characteristic dorsoventral arrangement from proliferating progenitors in the neuroepithelium.
Materials and Methods: To investigate how the growth, proliferation and differentiation of neural progenitors affect cellular dynamics and pattern formation we developed a 2D mechanical model of the dorsoventral and anterior-posterior axes of the neural tube, see [1]. This model represents the apical surface of neural progenitors as an epithelial junctional network in which the vertices of the network move in response to forces arising from interfacial tension, cortical contractility and cell pressure. To establish the model, we used experimental measurements from the embryonic mouse neural tube and incorporated neural tube specific features including inter-kinetic nuclear movement and spatially varying rates of neuronal differentiation.
Results: Comparison to experimental data indicated that the model captured the principal characteristics of cell geometry and tissue dynamics of the neural tube. Strikingly, the simulations predicted that the shape of lineage related clones of cells differ with the rate of neuronal differentiation. In regions of low differentiation, clonal shape was dorsoventrally biased. By contrast, in regions of high neuronal differentiation, clones were isotropic in shape. This difference recapitulates experimentally determined differences in neural tube clone shape: clones in rapidly differentiating motor neuron progenitors have equal dorsoventral and anteroposterior spread, whereas clones in slower differentiating dorsal neural progenitors are elongated along the dorsoventral axis. The absence of planar or long range signalling in the simulations indicates that mechanical constraints are sufficient to explain this behaviour without the need for additional molecular mechanisms.
Conclusions: The result provides insight into how tissue growth and cell dynamics are coupled in the neural tube and highlights how isotropic cellular processes, such as neuronal differentiation, can have directional effects at the tissue level. More generally, the availability of computational tools that accurately reproduce the cellular dynamics of the neuroepithelium opens up the possibility of developing realistic simulations that address how neural tube growth is coordinated with molecular pattern formation.
References:1 – P. Guerrero, R. Perez-Carrasco, M. Zagorski, D. Page, A. Kicheva, J. Briscoe, K. Page, **Neuronal differentiation influences progenitor arrangement in the vertebrate neuroepithelium**, Development Vol. 146, dev176297 (2019). D.O.I.: 10.1242/dev.176297.

56ASM-0317 S4 IS | A cellular Potts model describing wound healing assays in diverse cell motility and cell adhesion conditions

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Background: Cell motility is fundamental in many physiological processes in normal development, from embryogenesis to angiogenesis, but also in pathological ones, from cancer invasion to wound healing. The capacity of cells to move in a directed way, to build a structure with well-defined shape and structure, is determined by many of their properties, like adhesion and chemotaxis. It is fundamental to understand all these mechanisms as any failure has serious consequences for health.
Materials and Methods: We implemented a computational cellular Potts model (CPM) to describe a wound healing assay, a standard in vitro technique for testing and measuring collective cell migration in two dimensions [1].

After the removal of the barrier that separates two regions of cell culture, their movement to fill the gap is measured as a function of different cell properties, and compared with experimental results. In the CPM, each cell is described by a group of pixels, creating connected structures, and the system evolves by copy attempts, from each pixel on the cell surface to a neighbor one [2]. The copy acceptance is determined by the change of the system energy, which includes terms on the cell area, membrane perimeter, cell adhesion, chemotaxis and polarization.

Results: The model was used to study the wound healing assays performed with cells from the gastric pits, both wild type and with mutations on E-cadherin, a cell adhesion molecule important in cells adhesion to each other. The experimental results, including the cells trajectory, travelled distance and average velocity, and the gap closure progression, where described. From the simulation, were established the most important factors that determine their behavior for different mutations. A lower cell adhesion drives a greater cell motility, as they are freer to move, but also the response to chemotaxis is fundamental for the gap closure timing. Cells polarization, which determines their shape, is also a relevant factor in the wound healing results.

Conclusions: The results presented show that the CPM can reproduce well the experimental results and also point out the most important factors that determine cell motility. The cells loss of adhesion, as due to mutation of E-cadherin, present in the diffuse gastric cancer, is important, but also their chemotactic capacity. A computational model, concentrating in the most relevant factors, can be very useful to describe and explain the biological experimental results, and to propose new tests.

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56ASM-0014 | Kinetics of ficin molecules aggregation by dynamic light scattering

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Background: Ficin (EC 3.4.22.3) is a proteolytic enzyme used in cosmetology due to its pronounced properties to soften and restore the skin, stopping peeling, it has antihelmintic, antibacterial, hemostatic effects.

Materials and Methods: The sizes of ficin molecules were determined by dynamic light scattering on a Nano Zetasizer ZS instrument (Malvern Instruments). Backscattered light from a 4 mW (632.8 nm) He/Ne laser was collected at an angle of 173°.

Results: At a ficin solution concentration of 1 mg/mL, the average radius of the peaks corresponding to the native form of the enzyme almost completely coincides at 50, 60, and 70°C. At 80 °C, this peak is absent. The highest height of this peak with the smallest width is observed at 60°C, which probably indicates a higher number of native enzyme molecules at this temperature. In addition, a peak corresponding to autolysis products is recorded at this temperature. At 50 and 70°C, the peaks corresponding to native ficin molecules and their aggregates merge, which may indicate the formation of a transitional forms number. The number of individual peaks corresponding to aggregates of enzyme molecules is 1 peak at 50 and 60°C, 2 peaks at 70°C, and 3 peaks at 80°C.

During the incubation of a ficin solution with a concentration of 1 mg/mL at temperatures of 50 and 60°C, a decrease in the number of native enzyme molecules is observed against the background of an increase in the number of aggregates. Incubation of a ficin solution at 70°C shows changes in the number of particles of different radii in the system, which do not have a definite pattern. At 80°C, complete aggregation of particles in a solution of ficin with a concentration of 1 mg/ml is observed already at the beginning of incubation.

Conclusions: At 50, 60, and 70°C and a ficin solution concentration of 1 mg/mL, molecules corresponding in size to the intact form of the enzyme predominate. At 80 °C they disappear. In addition, at 60°C a peak corresponding to the products of enzyme autolysis is recorded.

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56ASM-0015 | Kinetics of papain molecules aggregation by dynamic light scattering

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Background: Papain (EC 3.4.22.2) is a plant proteolytic enzyme used for oral care as an additive that breaks down the protein base of dental plaque and thus softens it, helps to cleanse wounds, it is part of the enzyme biocleaning kit and to remove biofilms from substrates.
Materials and Methods: We determined the sizes of papain molecules by the method of dynamic light scattering using a Nano Zetasizer ZS device (Malvern Instruments). Backscattered light from a 4 mW (632.8 nm) He/Ne laser was collected at an angle of 173°.
Results: The average radius of the peaks corresponding to the native form of papain almost completely coincides at 60, 70, and 80°C. However, the intensity and band width in them differ, which may indicate both a different number of native enzyme molecules at the initial moment of incubation, and a difference in the degree of conformational lability and, as a result, polydispersity of enzyme forms. More pronounced aggregation processes are recorded at 80°C, which is also evidenced by the appearance of an additional aggregate peak.
During the incubation of a papain solution with a concentration of 1 mg/mL at temperatures of 60, 70, and 80°C, a decrease in the amount of the native enzyme is observed against the background of an increase in the number of aggregates. At all temperatures under study, aggregation processes are detected already from the beginning of incubation.
After one and a half hours of incubation of the papain solution, the intensity of the peak corresponding to the native form of the enzyme at 70°C exceeds that at 60°C, which is probably due to the precipitation of aggregates of the enzyme molecules at 70°C. At 60°C, an additional peak is detected, corresponding to large aggregates of papain molecules.
At 80°C, there is a quantitative predominance of enzyme molecules aggregates during the entire incubation time.
Conclusions: At 60, 70, and 80°C, different amounts of native papain molecules are detected at the initial moment of incubation, a difference in the degree of their conformational lability and polydispersity of enzyme forms are observed. The degree of particle aggregation also differs at different temperatures. Aggregation processes proceed more intensively at 80 °C.

The study was supported by a grant from the Russian Science Foundation (projectNo. 21-74-20053)

56ASM-0016 | Kinetics of bromelain molecules aggregation by dynamic light scattering

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Background: Bromelain (EC 3.4.22.4) is a proteolytic enzyme widely used in medical practice and cosmetology. Bromelain is used in cosmetic treatment to destroy pigmentation and scar tissue, is used to make a drug for the treatment and/or prevention of eye diseases.
Materials and Methods: We determined the sizes of bromelain molecules by dynamic light scattering using a Nano Zetasizer ZS device (Malvern Instruments). Backscattered light from a 4 mW (632.8 nm) He/Ne laser was collected at an angle of 173°.
Results: The average radius of the peaks corresponding to the native form of bromelain almost completely coincides at 50 and 60°C. However, the intensity and band width in them differ, which may indicate both a different number of native enzyme molecules at the initial moment of incubation, and a difference in the degree of conformational lability and, as a result, polydispersity of the enzyme forms at 50 and 60°C. At 50°C, a peak is recorded indicating the presence of autolysis products. The peaks corresponding to the enzyme molecules aggregates also almost completely coincide in radius with a slight difference in intensity. At 60 °C, an additional peak appears.
During the incubation of the bromelain solution at 50°C for the first 70 minutes, an increase in the number of native enzyme molecules is recorded against the background of a decrease in the number of aggregates. In parallel with this, the amount of autolysis products increases, the presence of which is recorded already from the beginning of incubation. However, after one and a half hours, the number of native enzyme molecules significantly decreases against the background of an increase in the number of aggregates. At the end of the incubation period, there is a significant predominance of aggregates.
Incubation of bromelain solution with a concentration of 1 mg/ml at 60°C shows a decrease in the number of native enzyme molecules against the background of an increase in the number of aggregates with complete aggregation of particles in the system under study after 40 min.

At 70°C, complete aggregation of particles in the system under study is observed already from the first minute of incubation.

Conclusions: At 50 and 60°C, differences are observed in the degree of conformational lability and, as a result, in the polydispersity of the forms of bromelain molecules. At 70°C, complete aggregation of the enzyme is observed from the first minute of incubation.

The study was supported by a grant from the Russian Science Foundation (projectNo. 21-74-20053)

56ASM-0018 | Virtual screening of immobilization agents for bromelain, ficin, papain among biodegradable polysaccharides modified with vinyl monomers

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Background: Bromelain (EC 3.4.22.4), ficin (EC 3.4.22.3), papain (EC 3.4.22.2) are cysteine proteases widely used in pharmacy and medicine. However, enzyme solutions have a number of limitations in their application, which can be overcome by their immobilization on insoluble polymer carriers. The advantages of immobilized enzymes in comparison with their soluble forms are as follows: increased stability of the medicine, the possibility of its targeted delivery and fixation in certain areas of the body, prolonged action of the active substance, therefore, a decrease in the concentration of the active substance in the body.

Materials and Methods: Bromelain (PDB ID: 1W0Q), ficin (PDB ID: 4YYW), papain (PDB ID: 9PAP), and graft copolymers of chitosan or carboxymethyl cellulose with N-vinylimidazole, N,N-dimethylaminoethyl methacrylate and 1-vinyl(3,5-dimethylpyrazole) with different content of vinyl comonomer units.

The preparation of the enzyme structure for docking was carried out according to the standard scheme for Autodock Vina. Models of polymer structures were drawn in the HyperChem molecular constructor, sequentially optimized first in the AMBER force field, and then quantum-chemically in PM3. Each of the ligands in the docking calculations had maximum conformational freedom: the rotation of functional groups around all single bonds was allowed. The arrangement of charges on the ligand molecule and its protonation/deprotonation was carried out automatically in the MGLTools 1.5.6 package.

Results: The binding energies of the studied polymers with bromelain, ficin, and papain were calculated. The lowest values of this indicator were found for bromelain, which may indicate a higher degree of affinity for the given carriers during immobilization. Papain has a degree of affinity similar to that of bromelain for Cht-*g*-P(VI-*co*-VDMP).

It has been established that all the polymers studied by us bind approximately in the same region on the surface of the molecules of bromelain, ficin, and papain, which is a groove. An exception is the site of Cht-*g*-P(VI-*co*-VDMP) binding to the ficin surface.

Conclusions: The method of flexible molecular docking revealed promising areas on the surface of molecules of bromelain, ficin and papain and active groups of a number of potential carriers for their immobilization on biodegradable polysaccharides modified with vinyl monomers, due to which enzymes are adsorbed.

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56ASM-0019 | Designing optimized drug candidates with generative adversarial network

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Drug design is an important area of study for pharmaceutical businesses. However, low efficacy, off-target delivery, time consumption, and high cost are challenges and can create barriers that impact this process. Deep Learning models are emerging as a promising solution to perform de novo drug design, i.e., to generate drug-like molecules tailored to specific needs. However, stereochemistry was not explicitly considered in the generated molecules, which is inevitable in targeted-oriented molecules.

This paper proposes a framework based on Feedback Generative Adversarial Network (GAN) that includes optimization strategy by incorporating Encoder-Decoder, GAN, and Predictor deep models interconnected with a feedback loop. The Encoder-Decoder converts the string notations of molecules into latent space vectors, effectively creating a new type of molecular representation. At the same time, the GAN can learn and replicate the training data distribution and, therefore, generate new compounds. The feedback loop is designed to incorporate and evaluate the generated molecules according to the desired property at every epoch of training to ensure a steady shift of the generated distribution towards the space of the targeted property. The results demonstrate that the proposed framework can generate realistic, novel molecules that span the chemical space. The proposed Encoder-Decoder model correctly reconstructs 99% of the datasets, including stereochemical information. The model's ability to find uncharted regions of the chemical space was successfully shown by optimizing the unbiased GAN to generate molecules with a high binding affinity to the Kappa Opioid and Adenosine A2a receptor. Furthermore, the generated compounds exhibit high internal and external diversity levels 0.88 and 0.94, respectively, and uniqueness.

56ASM-0020 | Flexible molecular docking to identify optimal binding sites and affinity energies of potential carriers for immobilization to papain

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Background: Papain (EC 3.4.22.2) is derived from *Carica papaya* latex. Apart from its protease activity, papain possesses amidase, esterase, transamidase, transesterase, and thioesterase activities.

Materials and Methods: Papain (PDB ID: 9PAP) as well as cellulose and chitosan derivatives were used as objects for *in silico* calculations. The preparation of the enzyme structure for docking was carried out according to the standard scheme for Autodock Vina. Models of polymer structures were drawn in the HyperChem molecular constructor. Each of the ligands in the docking calculations had maximum conformational freedom. The arrangement of charges on the ligand molecule and its protonation/deprotonation was carried out automatically in the MGLTools 1.5.6 package.

Results: Calculations showed that the vast majority of ligands bind in the region of the active center of papain, which is located on the side of the protein globule in the so-called "pocket". It should be noted that the larger and more branched the ligand molecule, the higher the probability that its side groups will go beyond the boundaries of this pocket and bind to amino acid residues on the surface of the globule.

When discussing the bonds that are responsible for the complexes stabilization of ligands with a papain molecule, we should first of all note hydrogen bonds. It is noteworthy that on the protein side, the amino acids of the active center are most often involved in such bonds, especially His159, as well as Gly20 and Gly66 (which do not have a side group and bind along the main protein chain). Aromatic residues in the globule play a dual role: for example, Tyr67 is able to participate in the formation of hydrogen bonds with ligands, also act as a hydrophobic partner. Hydrophobic interactions additionally stabilize the complex; Trp177, Trp181, and Thr204 are responsible for this in the protein.

Conclusions: Of the presented set of ligands, the most stable complex of the papain molecule with chitosan and its acetate was formed, while other modifications of polysaccharides demonstrated a decrease in the affinity of the ligand for the protein globule. In all cases, which is important, the binding occurred with the ligand overlapping the active site of the enzyme.

The work was partially funded by the internal grant of Sevastopol State University, Grant number: 42-01-09/90/2020-2 and by the program 'Prioritet-2030' of Sevastopol State University (strategic project No. 3).

56ASM-0021 | Flexible molecular docking to identify optimal binding sites and affinity energies of potential carriers for immobilization to bromelain

V. Koroleva[1]; M. Lavlinskaya[2]; A. Sorokin[2]; M. Holyavka[3]; S. Pankova[4]; S. Olshannikova[5]; M. Kondratyev[6]; A. Dubovitskaya[5]; T. Belyaeva[5]; Y. Redko[5]; N. Malyhina[5]; D. Bogomolov[5]; D. Paymetieva[5]; A. Pashkov[7]; V. Artyukhov[5]
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Background: Bromelain is extracted from stems (EC 3.4.22.32) and unripe fruit (EC 3.4.22.33) of *Ananas comosus* of the family Bromeliaceae. Fruit bromelain has higher proteolytic activity compared with its stem counterpart and has broader peptide bond specificity.
Materials and Methods: Bromelain (PDB ID: 1W0Q) as well as cellulose and chitosan derivatives were used as objects for *in silico* calculations. The preparation of the enzyme structure for docking was carried out according to the standard scheme for Autodock Vina. Models of polymer structures were drawn in the HyperChem molecular constructor. Each of the ligands in the docking calculations had maximum conformational freedom. The arrangement of charges on the ligand molecule and its protonation/deprotonation was carried out automatically in the MGLTools 1.5.6 package.
Results: Unlike the molecules of other thiol proteases, the bromelain globule has a larger cavity, with the walls of which the studied ligands bind. Depending on the size of the ligand, during the calculation, it either largely ended up in this cavity, or "clung" to its entrance with side groups. The most immersed and unbranched ligands are characterized by the highest affinity energy values are chitosan acetate and chitosan sulfate with -9.4 kcal/mol and -9.0 kcal/mol, respectively. 2-(4-Acetamido-2-sulfanilamide) chitosan has a more developed skeleton of the molecule and the whole cannot fit in this cavity, but it has the highest affinity with -9.5 kcal/mol.
Speaking about the role of hydrogen bonds in the stabilization of complexes, one should note the importance of the interactions of the –OH groups of the ligands with Lys18 and Lys179, and not with the side group, but with the oxygen and nitrogen atoms of the main chain. This rather unexpected phenomenon is also observed when the –OH groups of the ligands are bound to the Asn19 and Gly184 residues of the protein. Hydrophobic interactions in the complexes are carried out mainly due to the aromatic residues Trp180 and Tyr185.
Conclusions: Of the presented set of ligands, the most stable complex with the bromelain globule was formed by chitosan derivatives: 2-(4-acetamido-2-sulfanilamide)chitosan and chitosan acetate, while other modifications of the carrier demonstrated a decrease in the affinity of the ligand for the enzyme globule.
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56ASM-0022 | Design of conjugated ionic-hydrogen bonds between peptide sequences and sites of protein biomarker of non-communicable diseases

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Background: The aim of work is development of method for detection in biological fluids informative series of protein biomarkers enabling a systemic multiparametric diagnostics of a number of diseases. The theoretical background for the applied approach were the complementarity of the contact area of the subunits of oligopeptides and the formation of conjugated ionic-hydrogen bonds systems (CIHBS) during the formation of complexes "target protein - peptide aptamer".
Materials and Methods: The work was carried out using the visualizer of protein structures Protein 3D, used in analysis of the spatial structure of the contact areas of subunits of marker proteins (myoglobin, troponin, myeloperoxidase, S-100), and finding complementary regions in these areas based on CIHBS formation criterion for the formation of stable complexes between target protein and peptide aptamer".

Results: The structure of the horse myoglobin dimer proved to be the most suitable for creating an aptamer and it is similar enough to human myoglobin. In myoglobin dimer, α-helical regions are in the contact area of two subunits, and for human myoglobin, it was proposed to synthesize the following sequence: Leu-Thr-Ala-Leu-Gly-Gly-Ile-Leu-Lys-Lys-Lys-Gly-His-His-Glu-Ala-Glu-Ile-Lys-Pro-Leu-Ala-Gln-Ser-His-Ala-Thr-Lys-His-Lys. Troponins C, T and I form a complex as one subunit, while two subunits oriented at an angle to each other form a dimer. The analysis of the interaction of individual chains within the subunit, shows multiple contacts with two other troponins, and the molecules of troponin I and troponin T are in close contact with each other and have kinks in α-helixes. Thus, the promising sequence for using as troponin T-specific ligand is His-Leu-Asn-Glu-Asp-Gln-Leu-Arg-Glu-Lys-Ala-Lys-Glu-Leu-Trp-Gln-Thr-Ile-Tyr-Asn-Leu-Glu-Ala-Glu-Lys-Phe-Asp-Leu-Gln-Glu-Lys-Phe-Lys-Gln-Gln-Lys-Tyr-Glu. Myeloperoxidase (MPO) consists of two identical dimers connected by a disulfide bond, each of which contains a heavy α-chain with a covalently linked heme and a light β-chain. The following sequence of light chain can be recommended for creating a ligand: Ser-Leu-Met-Phe-Met-Gln-Trp-Gly-Gln-Leu-Leu-Asp-His-Asp-Leu-Asp. S100 proteins are a group of low molecular weight proteins. In this work, we analyzed a dimer with subunits that are in close contacts, and these contacts are symmetrical. It is proposed to synthesize the following sequence: Ser-Glu-Leu-Glu-Lys-Ala-Met-Val-Ala-Leu-Ile-Asp-Val-Phe-His-Gln-Tyr-Ser-Gly-Arg-Glu-Gly-Asp-Lys-His-Lys-Leu-Lys-Lys-Ser-Glu-Leu-Lys-Glu-Leu-Ile-Asn-Asn-Glu-Leu-Glu.
Conclusions: Based on the analysis performed, peptide fragments were proposed for using as microarray ligands for detecting the protein markers in biological fluids.
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56ASM-0028 | Method for assessing motor activity by the capture system

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Background: Comparison of the parameters of movement of healthy subjects and subjects with injuries is an urgent task of our time. The kinematic characteristics of the movement make it possible to describe the spatial displacements of the body and its individual links in space. Moreover, using kinematics data it is possible to solve the inverse dynamic problem to find forces that initiate these displacements. The purpose of this work is to develop and describe methods for assessing the movement of the subject.
Materials and Methods: During motion capture, the rats began to move naturally in an open field. Spline interpolation was used to resample Vicon data up to 30 Hz before analysis. Kinematic analysis was carried out for the complete gait cycle of each test Wistar rat. To analyze the data obtained, the described method was implemented in the MATLAB software package. Three-dimensional data was obtained using six Vicon MX cameras. To analyze angle changes results were averaged for all steps. In this case, the resulting distribution can be presented as follows: $\bar{\varphi}_{\pm}(\tau) = \text{mean}(\varphi(\tau),N_{step}) \pm \text{std}(\varphi(\tau),N_{step})$ where $\varphi(\tau)$ – angle function, mean (·, k) and std (·, k) – mean and standard deviation of function according to steps, N_{step} – number of steps.
Results: Calculated parameters such as step length, maximum foot lift, and foot swing give a clear indication about the subject moving. Also, the construction of angulograms is informative, the average values of the change in the angles of the hip and knee in steps and the standard deviation are calculated. Angulograms can be used to determine gait in normal and pathological conditions, as well as before and after treatment.
Conclusions: The developed technique helps to accelerate the diagnosis of the subject's disease and personalize the treatment. The calculated parameters are indicative and give a clear picture of the nature of the subject's movement. This technique will be useful for tracking the dynamics of the subject's state. The proposed methodology is based on simple optimal algorithms and is easy to be automized. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

56ASM-0034 | Semiflexible polymers under external fields

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Background: Semiflexibility is a peculiar property of several biological polymers. Examples are provided by DNA, filamentous actin, microtubules, or viruses. Their rigidity is relevant for their biological functions.

Materials and Methods: The non-equilibrium structural and dynamical properties of semiflexible polymers confined to two dimensions are investigated by Brownian multi-particle collision dynamics.
Results: Different scenarios are considered: tethered polymers subject to an external force, chains under steady shear flow [1], and filaments with either both [2] or one fixed end [3] under oscillatory shear flow. Experimental results are recovered and new predictions are provided when the filament is exposed to large amplitude oscillatory shear.
Conclusions: Our results show that the proposed method is useful in order to capture the relevant physical properties of semiflexible polymers under non-equilibrium conditions.
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56ASM-0059 | Polymer models of retroviral integration in DNA and genomes

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It is now well established that some classes of retroviruses, such as HIV, display highly non-random integration patterns along the genome, yet the underlying mechanisms driving this selection are poorly understood. Here I will present a recent effort to rationalise these observations via a generic multi-scale physical framework where HIV integration is modelled as a simple "reconnection" process between polymeric strands. Stochastic simulations and energetic considerations show that the large-scale 3D chromatin folding is one of the main drivers biasing the distribution of HIV integration sites towards euchromatic regions. Furthermore, a simple dynamical model explains the distribution of HIV hot-spots in human T-cells. These results are in very good agreement with experiments on HIV integration and strongly support the importance of polymer models in understanding the role of 3D genome organisation in many biological processes in the cell.

56ASM-0085 | Molecular dynamics simulation of human chromosome: cluster formation, transcriptional activity and correlation regulatory networks

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Background: Several studies proved a connection between chromosome spatial organisation and gene expression. This interplay is often analysed through molecular dynamics methods. We adopt a revised numerical approach to improve the predictive power of previously considered models.
Materials and Methods: We consider a fitting-free chain polymer modelling of chromatin fiber. Each bead represents a 3Kbp chromatin section. Some beads are identified as transcription units (TUs) and can be sites of transcriptional initiation. A set of spheres represents complexes of transcription factors and RNA polymerases (TFs). We consider three types of TFs that can bind specifically TU beads and non-specifically to every other bead and can switch between a binding and a non-binding state with a fixed rate. We focus on two chromatin fibers: a toy 3 Mbp chromatin fragment and the 107 Mbp human chromosome 14 in human umbilical vein endothelial cell (HUVEC). In the former case TU locations and colours are chosen randomly, in the latter they are identified using experimental data.
Results: We find TFs to bound in type-separated clusters (bridging-induced attraction, cluster segregation), despite no attractive interactions between TUs or TFs. We also measure the transcriptional activity, obtaining for HUVEC an optimal agreement between experimental (GRO-seq) and numerical data, as confirmed by a higher Spearman rank correlation than in previous models. Finally, we investigate the correlations between transcriptional activities of different TUs and identify an emergent regulatory network in which TUs form nodes: TUs in the same cluster tend to be active at the same time, result positively correlated and reduce the likelihood of cluster formation elsewhere.
Conclusions: In conclusion, not only we find good agreement between experimental and numerical data, but we also observe new features absent in previous models. Our improved model is then able to efficiently capture complex biological features and presents a higher degree of predictive power.

56ASM-0086 | Deep modelling for anti-cancer drug response through gene expression and mutation data

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Background: In the era of precision medicine, the main goal is to build a scenario where drug prescription is based on patient profile. However, due to tumor heterogeneity, drug response prediction for cancer therapy remains a challenge.
Materials and Methods: We propose a deep neural network model to predict the effect of anti-cancer drugs in tumors through the half-maximal inhibitory concentration (IC50). The dataset used includes tumors from The Cancer Genome Atlas Program (TCGA), human cancer cell lines from Cancer Cell Line Encyclopedia, and drug intolerance data from the Genomics of Drug Sensitivity in Cancer (GDSC) project.
The model can be seen as two-fold: first, we pre-trained two autoencoders with high-dimensional gene expression and mutation data from TCGA tumors to capture the crucial features and reduce data dimension; then, this genetic background is translated to cancer cell lines to predict the impact of the genetic variants on a given drug. For this purpose, we use GDSC data correlated to the genomic data to identify genetic features that are predictive of sensitivity. Given a set of mutation and expression profiles, the model predicts IC50 values of 265 drugs.
Results:

	Proposed Model	Random Forest	SVM
MSE	1.88	2.86	8.92
RMSE	1.36	1.69	2.99

Overall, the proposed model outperformed the classical methods, like Support Vector Machine (SVM). The results obtained reveal the model's potential once it demonstrates a correlation between predicted IC50 and the original data, i.e., the model is capable of extracting features from genomic data to improve drug response prediction.
Conclusions: The presented approach addresses the need for a translation of genomics features from tumor samples to predict the drug response of different types of cancer. This model integrates different types of omics data and, afterward, this knowledge will be combined with the chemical information present in the molecules so that the model can apprehend new relevant features.

56ASM-0089 | Dynamical properties of clusters of active particles

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Background: Active matter consists of self-propelled units that continually consume internal energy to self-propel and move in the environment. Examples include living organisms, such as bacteria or synthetic microparticles. As a result of their persistent motion, active particles show a tendency to arrange into clusters although the complete absence of attractive interactions. The aim of this work is to describe the nature and the dynamics of clusters formed by active particles. Understanding their behaviour would be of interest for numerous applications in medicine, biology and from a theoretical point of view.
Materials and Methods: We performed a numerical study using Molecular Dynamics (MD) simulations of a system of Active Brownian Particles (ABPs) in two spatial dimensions, which is a paradigmatic model of an active system particularly suitable to describe the motion of microscopical objects. We developed an algorithm to track individual clusters over time, in order to measure quantities related to their dynamical evolution.
Results: We found the formation of microdomains inside the clusters, corresponding to different orientations of the particle's packing, the size of which can be controlled by the intensity of the self-propulsion. We also provided a detailed analysis of the dynamics of individual clusters. We show that the clusters have a diffusive behaviour that is enhanced by the self-propulsion strength and that their diffusion coefficient depends in a non-trivial way on the total mass of the cluster. We explain this anomalous behaviour by the appearance of correlations at the edge of the clusters, which is an effect induced by the persistent motion of the particles.
Conclusions: Our study shows that the self-propulsion i) is able to trigger the formation of finite-size structures in a paradigmatic system, ii) dramatically influence the diffusion proprieties of the clusters, thus providing a substantial contribution to the understanding of active materials and the control of their self-organization.

56ASM-0122 | Perception of the target signal in conditions of contralateral interference in listeners with normal hearing and sensorineural hearing loss

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Background: A variety of interference masking the target speech signal makes it difficult to recognize speech even for normal hearing (NH) people. This problem is getting worse in patients with auditory disorders. The interaction between two signals can be evaluated by determining the threshold of the target signal in presence of interference.
Materials and Methods: 10 NH listeners and 24 patients with bilateral sensorineural hearing loss (SNHL), hearing aids (HA) users, were examined. Among the patients with SNHL 12 persons (the 1st group) did not have any signs of the central auditory processing disorders and 12 persons had these symptoms. The influence of the tonal contralateral interference (500 Hz) on the target tonal signal (1000 Hz) was evaluated according to the target signal threshold shift in conditions of presence of interference comparing to its threshold in quiet (Δ dB). The measurements were carried out with the use of a specially designed software-hardware complex.
Results: In NH listeners Δ dB increased with rising intensity of contralateral interference, and in cases of maximum intensity did not exceed 11.5 dB ± 3.7 dB, with the sentence intelligibility being 16.1 ± 3.2 dB in quiet and -9.2 ± 0.6 dB SNR (Signal-to-Noise Ratio) in noise. In patients with SNHL of the 1st group Δ dB did not differ from normal ones and did not exceed 11.7 ± 6.9 dB. In the 2nd group of patients Δ dB values reached 32.3 ± 12.9 dB, while the speech intelligibility using HA was lower than in the 1st group. In the 2nd group only in the right ear a correlation between the speech intelligibility and the Δ dB was revealed, which is the evidence of poor processing of speech signals.
Conclusions: The measurement of the target signal threshold shift in conditions of contralateral interference might be recommended as a method to predict HA benefit.
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56ASM-0123 | Approaches to the analysis of objective tinnitus

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Background: Objective tinnitus can be heard not only by a patient, but also by others. Such types of tinnitus are extremely rare and can be of a muscular origin, a vascular origin, of a tubal genesis; less frequently they occur due to other reasons.
Materials and Methods: 19 patients aged from 21 to 69 years old with complaints of tinnitus were examined. All patients underwent ENT examination, auscultation of the external auditory canal to confirm the objective nature of the tinnitus. In addition, palpation of the temporomandibular joints and assessment of the mobility of the lower jaw were performed. If the muscular nature of the noise was suspected a transnasal examination of the nasopharynx with a flexible endoscope was performed since palatal muscles clonus may disappear during a pharyngoscopy. The audiological assessment included pure tone audiometry, impedancemetry, speech audiometry in quiet, registration of evoked and spontaneous otoacoustic emissions. Analysis of the acoustic characteristics of objective tinnitus was performed for all patients using a specially designed instrumental and methodological complex.
Results: Depending on the origin of tinnitus, the patients were distributed as follows: 9 had muscle tinnitus; 6 had tubal tinnitus; 3 vascular one; one female patient with bilateral mild high-frequency sensorineural hearing loss had a bilateral constant high-frequency monotonous tinnitus clearly auscultated near the auricle. The new technique makes it possible to establish the objective nature of tinnitus, to assess its intensity, spectral and temporal characteristics, which can serve as a basis for making a preliminary diagnosis and determining a plan for further management, saving the results in order to monitor the patient's condition.
Conclusions: The reasonability of examining patients with objective tinnitus using a special instrumental and methodological complex has been shown. Analysis of the acoustic characteristics of objective tinnitus provides additional information to clarify a possible cause of the tinnitus and further patient management.

56ASM-0126 | Assessment of the effect of complete and incomplete spinal cord injury on the mechanical properties of bone tissue

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Background: The study of the injured state of patients and their individual organs is an urgent task. The purpose of the work is to statistically evaluate the effect of spinal cord injury on the mechanical properties of rat bones.
Materials and Methods: All tests were conducted on nonlinear laboratory rats. The incomplete spinal cord injury (iSCI) with contusion was applied at the level Th7-Th8 so that damage to the sensory and motor axons lead to impaired hind limb function. A complete spinal cord injury (cSCI) with transverse spinal cord transection at the Th8-Th9 level was also reproduced. All experiments were performed according to bioethical standards and were approved by the local ethical committee of the Kazan Federal University. Full-scale tests of three-point bending were performed. The analysis of the results was carried out according to the values of the elasticity modulus (EM) and ultimate strength (US).
Results: The results were compared with the control group. It was shown that cSCI and iSCI increase the EM by 18% ($p < 0.1$) for femurs and decrease by 18% ($p < 0.1$) – for tibiae; cSCI decreases the EM of tibiae by 11% ($p < 0.1$); cSCI and iSCI decrease the US of femurs by 37% ($p < 0.05$) and 16% ($p < 0.1$), respectively; cSCI and iSCI decrease the US of tibiae by 10% ($p < 0.1$) and 17% ($p < 0.1$), respectively.
Conclusions: The results represent that cSCI and iSCI decrease the EM and US of femurs ($p < 0.1$), cSCI decrease the US of tibiae ($p < 0.05$).
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56ASM-0128 | An automated method for assessing bone strength based on CT data

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Background: The purpose of the study is to implement a method for numerically estimating the strength of inhomogeneous porous structures based on a three-dimensional isoparametric finite element (FE) of a continuous medium constructed according to computed tomography (CT) data.
Materials and Methods: CT-based digital prototype data can be used for weighted integration of the local stiffness matrix. This approach makes it possible to directly take into account the volumetric distribution of material properties. The computational mesh can be constructed by removing elements with a low bone content. The experiment was performed on the anterior bone of a Vietnamese swine. Validation of the method was based on the calculation of a normalized energy error. Full-scale test of three-point bending with rigid fixing of the proximal sections was performed.
Results: The maximum of the normalized energy error corresponded to the boundary FEs with a low bone fraction. The minimum energy error (5.9%) and the maximum Mises stress (430 MPa) related to the loaded area. The formation of a crack in the bone diaphysis can be explained by the maximum values for the first component of the principal stresses (390 MPa) and the minimum values – for the third (-430 MPa). The calculation time was about 15 minutes.
Conclusions: The study considers one of the possible approaches to numerical modeling of inhomogeneous porous media. The three-point bending experiment were carried out on a swine's anterior bone. The proposed approach leads to lower computational costs and simplifies the process of constructing a computational grid.
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56ASM-0136 | Single-nucleotide polymorphisms of POLQ rs3218634 and rs532411 may be associated with the risk of luminal breast cancer

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Background: Translesional DNA polymerase θ is encoded with the *POLQ* gene contributes to DNA synthesis associated to repair of DNA double-strand breaks. Recent studies have shown that *POLQ* upregulation predicts a poor prognosis in breast cancer. However, the role of *POLQ* genetic polymorphisms in breast cancer susceptibility remains poorly understood. The current study aimed to assess single-nucleotide polymorphisms (SNPs) in *POLQ* gene and their association with the risk of developing breast cancer.

Materials and Methods: Breast cancer patients ($n = 67$) including hereditary (HBC) ($n = 46$) and sporadic (SBC) breast cancer ($n = 21$), supplied with clinical information, and healthy volunteers ($n = 36$) were enrolled in the study. Tumor subtype was classified into two groups; triple-negative breast cancer (TNBC) ($n = 27$) and luminal breast cancer ($n = 36$). Genomic DNA was isolated from peripheral blood mononuclear cells. The sequencing was performed with custom enrichment panel. The odds ratio, 5 % confidence interval, and *p*-value were determined applying Fisher's exact test.

Results: The distribution of the frequencies of the 12 POLQ SNPs rs487848, rs3218634, rs702017, rs532411, rs1381057, rs3218649, rs3218651, rs139982859, rs41540016, rs3218636, rs2306211, rs3218642 among patients with TNBC, luminal breast cancer and control group were estimated. Two POLQ SNPs rs3218634 (p.Leu2538Val) and rs532411 (p.Ala2304Val) were detected in 21.1% of patients with luminal BC, 6,5% of HBC and 19,0% of SBC while in the control group the frequency was 2.8% (OR = 9.3; 95% CI 1.22 - 106.1; $p = 0.003$), polymorphisms were not detected among patients with TNBC. This SNPs are in high linkage disequilibrium in European population and lead to the structure rearrangement of DNA polymerase θ.

Conclusions: POLQ SNPs rs3218634 and rs532411 may be associated with an increased risk of developing luminal breast cancer.

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56ASM-0226 | Timing treatment: profiling the circadian clock and optimizing treatment timing in cancer

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Background: The biological clock (circadian clock) regulates several aspects of physiology and behaviour and plays a vital role in human health. About half of all human genes are rhythmically expressed in at least one tissue, and the time for certain activities such as sleep, exercise, or medicine intake, can be optimized based on the internal circadian rhythm. Recent studies, including work from our group have highlighted a role for clock dysregulation in several hallmarks of cancer related to cell cycle, apoptosis or metabolism. Thus, scheduling anticancer drug administration over 24h may critically impact treatment success in a drug- and patient-specific manner.

Materials and Methods: We used a computational approach to generate a merged ODE (ordinary differential equation) mathematical model of the core-clock and pharmacology for irinotecan, an anticancer drug widely used against digestive malignancies.

Results: Our mathematical model, enables predictions of the timing-dependent cytotoxicity of irinotecan having as an input the circadian gene expression profiles of a set of core clock and irinotecan-related mRNAs. The mathematical model is fitted to different scenarios, including human colorectal cancer cell lines representing an in vitro model for colorectal cancer progression. Our model successfully recapitulated quantitative circadian datasets of mRNA expression together with timing-dependent irinotecan cytotoxicity data. The model also discriminates time-dependent toxicity between the different cells, suggesting that treatment can be timely optimized to allow for less toxicity in healthy cells and more toxicity in tumour cells.

Conclusions: Our results suggest that, in addition to clock and pharmacological gene expression, the circadian dynamics of translation and cell death, plays an important role in the timing of drug toxicity. Systems biology approaches addressing the personalization of cancer chronotherapies are needed for patient benefit, and such model can be used to support personalized treatment scheduling,

by predicting personalized drug toxicity based on the patient's gene or protein expression profiles.

56ASM-0228 | Transcriptome analyses of circadian clock disrupted cancer cells reveals differential alternative splicing of cancer hallmarks genes

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Background: Emerging evidence points towards a regulatory role of the circadian clock in alternative splicing (AS). Whether alterations in core-clock components contribute to differential AS events is largely unknown.
Materials and Methods: We carried out a computational analysis on recently generated time-series RNA-seq datasets from three core-clock knockouts (*ARNTL*, *NR1D1*, *PER2*) and WT of a colorectal cancer (CRC) cell line, and time-series RNA-seq datasets for additional CRC and Hodgkin's lymphoma (HL) cells, murine WT, *Arntl* KO, and *Nr1d1/2* KO, and murine SCN WT tissue.
Results: The deletion of individual core-clock genes resulted in the loss of circadian expression in crucial spliceosome components such as *SF3A1* (in $ARNTL^{KO}$), *SNW1* (in $NR1D1^{KO}$), and *HNRNPC* (in $PER2^{KO}$), which led to a differential pattern of KO-specific AS events. All $HCT116^{KO}$ cells showed a common rhythmicity loss of a crucial spliceosome gene *U2AF1*, which was also not rhythmic in higher progression stage CRC and HL cancer cells. AS analysis revealed an increase in alternative first exon events specific to *PER2* and *NR1D1* KO in HCT116 cells, and a KO-specific change in expression and rhythmicity pattern of AS transcripts related to cancer hallmarks genes including *FGFR2* in HCT116_$ARNTL^{KO}$, *CD44* in HCT116_$NR1D1^{KO}$, and *MET* in HCT116_$PER2^{KO}$. KO-specific changes in rhythmic properties of known spliced variants of these genes (e.g. *FGFR2* IIIb/*FGFR2* IIIc) correlated with epithelial-mesenchymal-transition signalling.
Conclusions: Altogether, our bioinformatic analysis highlights a role for the circadian clock in the regulation of AS, and reveals a potential impact of clock disruption in aberrant splicing in cancer hallmark genes.

56ASM-0246 | Trustworthy cardiovascular risk assessment

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Background: In spite of the remarkable achievements of artificial intelligence, the effective support to the clinical decision is still limited. The development of trustworthy cardiovascular risk assessment models is a key aspect to change this evidence, so explainability, personalization and reliability issues must be incorporated in the models' development.
Materials and Methods: The development of an inherently interpretable model typically faces the problem of lack of predictive performance. The application of a limited number of rules to a general population, different individuals, usually does not have an acceptable performance. This approach addresses this problem assuming that the same set of interpretable rules should not be applied in the same way to all individuals. Thus, individual rules are created based on the centroids of each class (event/no event). Each rule is evaluated (incorrect/correct) for each patient in the training dataset, providing a classification of all rules for all patients. Then a machine learning model is trained to select the subset of rules that has more potential to fit a specific individual. This perspective considers a machine learning model to identify the best set of rules to a new patient. The ensemble of the outputs of the selected rules provides the final classification of a new patient. Additionally, this approach provides a degree of confidence of the performed assessment for a given patient.
Results: The proposed approach was applied to patients with an acute coronary syndrome (risk of mortality/30 days). A dataset of $N = 1111$ patients provided by two Portuguese hospitals was considered. It achieved competitive performances when compared with black-box models as well as with the GRACE model.
Conclusions: This methodology implements a personalized scheme, explainable to some extent and it provides a reliability assessment. These aspects contribute to increase

the trust of physicians and consequently to promote the effective application on the support to clinical decision.

56ASM-0261 | Synthetic data augmentation for biological datasets

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Background: Machine Learning (ML) models have been playing an important role in extracting knowledge about complex systems such as biological systems. However, biological datasets tend to be small, which makes the application of ML models difficult. In this work, we used Deep Learning models to artificially generate new data samples.
Materials and Methods: Variational Autoencoders were used to obtain new datasets by training the generative models on real datasets with N samples and outputting N new records. To obtain high-performing models we executed fine-tuning with early stopping, optimizing the hyperparameters of the neural networks for different datasets of different sizes. Having the newly synthesized datasets, we proceeded to validate the data, comparing the real datasets (T_{orig}) with the synthetic ones (T_{syn}). The generated data was evaluated through a statistical lens and the utility of the data.
Results: To statistically validate our data we computed the Similarity, through the Kolmogorov-Smirnov (or Chi-Square) dimension-wise similarly and plotted the heatmaps of the Pearson's correlation (or Spearman's rank-order correlation) values, which allowed us to assess distributional differences between T_{syn} and T_{orig} and visually inspect the integrity of feature relationships. The best models were validated for their utility by training several classifiers in T_{orig} and T_{syn} and measuring their performance when predicting a target column. Our results in preliminary datasets showed that the accuracy and F1-score values achieved in both datasets were similar.
Conclusions: The ability of the synthetic data to maintain the utility and the statistical properties of the original data showcases the potential of Deep Learning to synthetically augment datasets, thus allowing to overcome the limitations of small data.
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56ASM-0309 | Endothelial cell dynamics in 3D vessel-like structures: a multi-phase field model

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Background: The circulatory system of animals is constituted by a complex network of blood vessels, whose main function is to transport nutrients and oxygen to cells and to carry out part of the cells' waste. Endothelial cells line the interior of blood vessels. Endothelial cells are very dynamic, as they change their phenotype as consequence of their interaction with other endothelial cells and with their surroundings, even responding to mechanical cues exerted by the blood flow.
Materials and Methods: We implement a computational multi-phase field model to simulate a group of cells [1] arranged in a tubular structure. Each cell is described by an order parameter field and their interactions with other cells, the extracellular matrix, and other agents, are introduced in the model via a free energy functional that determines the dynamics of the order parameters.
Results: We used this model to study two important processes related to endothelial cell organization and migration: endothelial cell polarization with flow in a blood vessel and sprouting angiogenesis. In the first scenario, we show that we can simulate the endothelial cell polarization and shape in a vessel by assuming that endothelial cells migrate in the blood flow direction with variable velocities. Their shape is strongly dependent on cell-cell and cell-matrix adhesion forces. We also show that when a single cell acquires the tip cell phenotype, it can prompt the growth of a sprout-like structure by recruiting its neighboring cells. This new sprout can be the start of a new blood vessel formed from the preexisting tube.
Conclusions: The results presented show that our model simulates important physiological processes using simple mechanisms that are well described in literature. Despite its simplicity, the model's complexity can be increased to account for other relevant physiological phenomena allowing us to study more complex processes.
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56ASM-0310 | Effect of anti-VEGF presentation in controlling vascular development

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Background: The progression of a large number of pathologies, such as tumor development and diabetes, is dependent on the development of new blood vessels. Glioblastoma multiforme (GBM), in particular, is characterized by a dramatic metabolic imbalance leading to increased secretion of the pro-angiogenic factor VEGF and subsequent abnormal tumor vascularization. In 2009, FDA approved the intravenous administration of bevacizumab, an anti-VEGF monoclonal antibody, as a therapeutic agent for patients with GBM.
Materials and Methods: In this work, we show that computational modeling can be used to quantitatively understand how the dynamics of VEGF secretion and diffusion, and subsequent vessel growth, depend on bevacizumab presentation. The implemented mathematical model of vessel growth is able to capture the resilience of neo-vascular networks' morphology.
Results: We have engineered a VEGF nanotrapper, a cargo system that allows cellular uptake of bevacizumab and strongly inhibits VEGF secretion required for angiogenesis activation and development. Here, we show the therapeutic efficacy of this nanocargo in reducing vascularization and tumor cell mass of GBM in vitro and in vivo cancer models. Moreover, the computational model highlights the possibility for an increase in vascular tortuosity in low VEGF conditions as a consequence of an alteration in the balance between different VEGF isoforms in the tissue.
Conclusions: The results presented demonstrate the capacity for mathematical modeling of angiogenesis to provide insight into the mechanisms behind VEGF diffusion and accumulation in the tissue, and consequent vascular development.
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SYMPOSIUM 5: CLINICAL ULTRASONOGRAPHY: TIPS & TRICKS

56ASM-0294 | How to become a Good Clinical Ultrasound Expert

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In recent years, we have seen an increasing use of bedside ultrasound in clinical practice as a novel kind of ultrasound stethoscope. To became a clinical ultrasound expert and increase the diagnostic capabilities are necessary good imaging skills and extensive medical knowledge. Other few important tips will help the clinician to became a master of ultrasound. Before to star scanning the clinicians must be sure and know well what they are looking for. It's important to talk to the patient, get more information from the referring physician, read charts and look at studies from other imaging modalities. Of course one should always perform a "complete" exam. But to use ultrasound to increase the diagnostic capabilities is better to search for something specific. Avoid to jump to conclusions too quickly. We see some or a pathology and already think we know what it is. But it is necessary to be careful: the laws of statistics state that even the improbable must occur sometimes. Observe first, then provide a detailed description of the ultrasound findings (size, location, echogenicity etc.) and finally make the interpretation. Interpreting sonographic findings is usually not a simple black and white issue. Don't be afraid of words such as "probably", "could be", "might be", "unclear" or "it appears". Such terms are important since they state a level of uncertainty. In any case ask others and your competence will increase. It is the power of "collective wisdom" that has led to such rapid developments in science. Research and follow the outcomes of the patients help to close the "loop of learning" to became a good clinical ultrasound expert.

56ASM-0313 | Clinical Ultrasonography: Tips & Tricks New Insights into Contrast Enhanced Ultrasound

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Brightness-mode (B-mode) US remains the mainstay for anatomical imaging of many organs and tissues but several new techniques have been introduced, among which contrast enhanced (CEUS) modes for vascular assessment.

CEUS is the most economically appropriate second-line imaging modality for the characterization of focal liver lesions after inconclusive baseline US in nononcologic noncirrhotic patients. In cirrhotic patients, CEUS allows characterization of contrast enhancement patterns of HCC with good sensitivity and specificity, with a much higher temporal resolution than CT or MRI . CEUS can also be used to guide locoablative therapies and to assess treatment response. In oncologic patients, CEUS provides higher sensitivity compared to unenhanced US for the detection of liver metastases and for characterization of liver lesions deemed indeterminate on CT and MRI. In patients treated with antiangiogenic therapies for solid tumors, data show encouraging results in the use of dynamic CEUS to distinguish responders from non-responders. In addition, microbubbles are being actively studied for local drug delivery under US triggering in animal studies.

New insights have also emerged concerning the use of microbubble ultrasound contrast outside the liver for the study of focal and inflammatory lesions in many systems and organs: genitourinary (bladder, kidney, Vesicoureteral Reflux, scrotum, prostate cancer, Transplanted Kidney, adrenal glands), Obstetrics and Gynecology, pancreas, gastrointestinal tract (including inflammatory bowel disease), spleen, Peripheral and cerebral Vascular System and Aorta, endoscopic (Contrast-Enhanced Endoscopic US, CE-EUS), abdominal trauma, superficial structures (thyroid, lymph nodes, salivary glands, breast), Inflammatory joint diseases, gallbladder diseases, neurosurgery, interventional CEUS, lung, pediatrics disease.

Intravenous and intracavitary CEUS use is safe and effective in both adult and pediatric populations. For the clinical implications of CEUS, the operator must gain sufficient knowledge and training in CEUS, ultrasound contrast agent administration and contraindications, and perform the examination within the medico legal framework of each individual country.

56ASM-0271 | Robust hepatorenal index algorithm using machine learning: a pilot study

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Background: Ultrasonography (US) is widely used to diagnose fatty liver, in particular by comparing Liver-to-Kidney echogenicity (i.e., hepatorenal index, HRI). The time-consuming and operator-dependent classical technique used for the manual calculation of HRI might limit its application in daily practice. Our study is therefore focused on defining a robust algorithm for HRI, able to increase accuracy and to reduce time, as well as operator and US machine dependency.

Methods: A robust algorithm was developed using not supervised machine learning (ML) techniques. The operator selects the regions of interest (ROI) on US images (Hitachi Noblus, Italy), and the software extracts the ROI identifying Kidney and Liver applies enhancements, balance filters and segmentation; HRI is therefore calculated considering pixel mean intensity and relative area of the segmented ROI. A dataset of US Liver/Kidney images from subjects with normal or mild fatty liver by three US machines and different operators served to validate efficiency, sensitivity, and accuracy of the algorithm.

Results: We compared results obtained from the algorithm with the HRI calculated manually from 3 expert operators using three different US machines. The comparison showed a significant linear correlation ($R = 0.89$ $P < 0.000001$) between the manually assessed HRI index and the ML algorithm. The algorithm was able to calculate the HRI in 1.5- 7 seconds, depending on image quality, dimension and ROI positioning. Results are characterized by a mean absolute error of 6% and a standard deviation of 4%, using manual HRI as reference.

Conclusions: The HRI obtained from the developed algorithm is a sensitive and noninvasive indicator of hepatic fat in subjects with mild-to-moderate liver steatosis. Data indicate that the algorithm is reproducible and may be operator- and machine-independent. This finding suggest that the developed algorithm could be used in medical field as an efficient diagnostic tool.

56ASM-0295 | Monitoring Gastrointestinal Motility in Response to Complex Mediterranean Foods (Gluten-Free Pasta) in Healthy Consumers

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Background: Functional foods produce a positive impact on digestive functions and metabolic profile, and the food industry is involved in assembling innovative formulations

of "Italian" pasta containing alternative wheat (legume) flours. These formulations, however, must be carefully explored in terms of gastrointestinal motility. Both functional ultrasonography and the H2-Breath test contribute to profiling individual dynamics in responses to complex foods. The aim of the study was to explore the acute effects of four different types of pasta/legume combinations on gastrointestinal motility in healthy subjects.

Methods: Forty volunteers ingested four different types of cooked pasta (80 g garnished with 13 g extra virgin olive oil, $N = 10$ subjects/group). The pasta was based on: 1) red lentils; 2) green beans; 3) buckwheat; 4) rice/corn (Andriani SPA-Natural innovators for conscious food, Gravina in Puglia, Italy). At baseline (fasting) and every 30 min for 2 hours after ingestion of pasta, we measured gastric and gallbladder emptying by functional ultrasonography (*Noblus* Hitachi and 3.5 MHz convex probe, Eurisko, Modugno, IT), and estimated oro-caecal transit time by lactulose H2-breath test (*Gastrolyzer* Bedfont Analyzer, Medimatica, Milano, IT).

Results: The speed and extent of postprandial gastric and gallbladder emptying were comparable among the four subgroups. Bean-containing pasta induced a shorter oro-cecal transit time (59 ± 8.7 min) than buckwheat and rice/corn pasta (91.2 ± 11.9 min vs 85.7 ± 7.2 min, respectively, $P = 0.047$). Postprandial levels of expired H2 as surrogate biomarker of gut microbiota fermentation of ingested carbohydrates were higher in response to pasta red lentils and green beans than buckwheat and rice/corn.

Conclusions: Both functional ultrasonography and the H2-Breath test provide time-dependent real proofs of combined gastric, gallbladder, and small intestine kinetics in response to complex foods. All types of pasta generated comparable gastric and gallbladder postprandial response, although a more rapid oro-cecal transit was observed in the case of pasta with green beans. Further studies will indicate the effects of these different types of pasta on gastrointestinal symptoms, metabolic indices and variations in gut microbiota.

56ASM-0259 | Body and liver fat accumulation increases liver stiffness independently from fibrosis

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Background: Fat accumulation can alter the mechanical phenotype of the liver. Increased liver stiffness, however, is also linked with fibrosis, potentially increasing disease severity from steatosis to steatohepatitis, and ultimately cirrhosis. It is still unclear if fat accumulation *per se* increases liver stiffness independently from fibrosis.

Materials and Methods: A total of 60 consecutive outpatients underwent Acoustic Radiation Force Impulse shear wave elastography of the liver (ARFI, liver stiffness), APRI and FIB-4 calculation. Ultrasonography (US) served to grade steatosis: absent/mild (normal liver echogenicity or isolate finding of liver echogenicity brighter than the renal cortex), moderate (additional presence of portal margin blurring), or severe (additional presence of diaphragmatic attenuation).

Result: Absent/mild (controls), moderate or severe steatosis was found in 40, 11 and 9 subjects, respectively. Subgroups were comparable for age and gender ratio. The body mass index (BMI, Kg/m2) was significantly higher in moderate (32.4 ± 1.4) or severe (34.3 ± 1.4) steatosis, than in controls (26.7 ± 0.7). Liver stiffness increased from 1.32 ± 0.04m/s in controls to 1.52 ± 0.08m/s and 1.58 ± 0.09m/s in moderate and severe liver steatosis, respectively ($P = 0.01$ ANOVA). Overall, ARFI showed a normal or mild (i.e., F1-F2) grade of fibrosis in 93% of subjects. The absence of severe fibrosis was confirmed by APRI (< 0.7 in all subgroups) and FIB-4 values (< 0.45 in all subgroups).

Conclusions The combined use of liver US and ARFI can reliably detect early alterations of the viscoelastic properties of liver tissue in subjects with NAFLD, in the absence of advanced fibrosis. In these subjects, increased liver stiffness seems to mainly depend on the extent of fat accumulation. This evidence might lead to primary and secondary prevention measures, able to avoid a possible progression towards more severe liver diseases.

56ASM-0174 | The Gut-Liver Body Axis & Lifestyles: Lessons from Frontline Biomedical Research

Aquaporins as emergent drug targets for obesity and cancer

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Since the discovery of aquaporins that research related to these membrane channels has never ceased growing. The classical water channels were found to have a much broader selectivity than initially expected, facilitating the transmembrane diffusion of many other small molecules, ions, and gases, and to be modulated by unique mechanisms allowing fine-tuning of their activity. In addition, aquaporin participation in cellular processes such as cell proliferation and migration highlighted their importance and their role as essential channels for life. A vast array of human disorders has been correlated with aquaporin dysregulation, missorting or mutations, unveiling their essential role in health and disease. The crucial role of aquaporins in kidney disease, brain oedema, metabolic disorders, obesity and cancer are examples of diverse pathological conditions where these proteins play a role. Here we present a few examples where targeting aquaporins may represent innovative therapies, unveiling their potential as drug targets for treatments and biomarkers for prognostic. Among the broad range of diseases where aquaporins are implicated, we are mostly interested in investigating their druggability for metabolic disorders (obesity, diabetes)1, cancer2, and inflammation3, 4, aiming to provide a basis for the development of aquaporin-based therapeutics for these diseases.

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SYMPOSIUM 6: MEMBRANE TRANSPORTERS AND CHANNELS: TRANSLATING BASIC RESEARCH TO NEW DRUG DISCOVERY AND PRECLINICAL DEVELOPMENT

56ASM-0050 S6 IS | Funny channel block by ivabradine: molecular views of cardiac therapies

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In cardiac pacemaker cells, activation of the so-called "funny" (I_f) current at the termination of an action potential controls initiation of diastolic depolarization, a crucial process in the generation of spontaneous activity. The funny current also modulates heart rate by controlling the steepness of diastolic depolarization, a main mechanism of heart rate control by the autonomic nervous system.
Because of their properties, f-channels have been a major target in the search for drugs able to specifically control heart rate. In particular, it is known that increased heart rate has a negative impact on clinical outcome in patients with cardiovascular disease, and is an established risk factor for cardiovascular and all-cause mortality in the general population. Thus, substances able to bind specifically to and *inhibit* funny channels can become useful pharmacological tools for heart rate reduction.
Of several funny channel-blocking drugs synthetized, ivabradine is the only heart rate-reducing substance approved for clinical use in Coronary Artery Disease and heart failure. Ivabradine specifically blocks funny channels from the cytoplasmic channel side. By reducing the I_f-regulated steepness of diastolic depolarization in SAN pacemaker myocytes, ivabradine slows heart rate in a dose-dependent way. Its block of I_f is selective and its action thus translates into specific heart rate reduction, with little cardiovascular side-effects. Since in physiological conditions funny channels are not expressed in the ventricles, use of ivabradine preserves ventricular contractility.
The molecular basis of the drug blocking action has been investigated in HCN4 channels, the α-subunits of native funny channels, and residues lining the channel pore

and interacting with ivabradine have been identified by cellular electrophysiology and docking studies. Recently, Cryo-EM resolution of HCN4 channels has confirmed the localization of the channel blocking site of ivabradine, providing a real-view snapshot of molecules docking in the water-filled cavity below the channel pore.

56ASM-0120 S6 IS | From mutations to drugs in ion channelopathies: precision medicine in non-dystrophic myotonia

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Nondystrophic myotonias (NDM) are rare diseases characterized by skeletal muscle stiffness due to sarcolemma hyperexcitability, which may greatly affect quality of life. NDM are caused by gain-of-function mutations of the Nav1.4 sodium channel or loss-of-function mutations of the ClC-1 chloride channel. Regardless the culprit gene, sodium channel blockers have been empirically used in myotonia because they reduce abnormal action potential firing in myofibers. Randomized clinical trials confirmed mexiletine effectiveness in myotonic patients. Yet about 30% of patients are intolerant or report unsatisfactory response to mexiletine. Alternative drugs are required to address the unmet needs of myotonic patients.

We hypothesized that drugs able to correct selectively the molecular defect of channel mutants would be greatly beneficial. Regarding sodium channel myotonia, myotonic Nav1.4 mutations can modify channel sensitivity to mexiletine, due to alteration of binding site or channel gating. In particular, we found that the Nav1.4 mutants with a positive shift of fast inactivation voltage dependence are less sensitive to mexiletine but conserve their sensitivity to the antiarrhythmic flecainide. Patients carrying such mutations and refractory to mexiletine were successfully treated with flecainide, which demonstrates the translatability of in vitro data to individuals. In chloride channel myotonia, ClC-1 loss of function stems from gating alteration or intracellular trafficking impairment. No direct ClC-1 channel activator is currently available. We used potent and reversible ClC-1 inhibitors to define binding sites and better understand effects on channel gating. In parallel, we performed proof-of-concept studies to verify the ability of pharmacological chaperones to restore sarcolemma expression of trafficking-deficient ClC-1 mutants. Altogether, these studies define a pharmacogenetics strategy to address precision medicine in myotonic individuals. Such an approach may serve as a paradigm for ion channelopathies. Supported by Italian-Telethon, Association Française contre les Myopathies, and University of Bari (project Medineuropa).

56ASM-0174 S6 IS | A challenging case of visceral leishmaniasis

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The term leishmaniasis includes multiple clinical syndromes: visceral, cutaneous, and mucosal leishmaniasis, which result from infection of macrophages throughout the reticuloendothelial system, in the dermis, and in the naso-oropharyngeal mucosa, respectively[1]. The clinical phenotype is mainly driven by the leishmania biologic characteristics and, ultimately, also by the host immune status. The disease is endemic in focal areas in the tropics, subtropics, and southern Europe, transmitted by the bite of female phlebotomine sandflies. Sandflies regurgitate the parasite's flagellated promastigote stage into the host's skin; promastigotes bind to receptors on macrophages, are phagocytized, and transformed within phagolysosomes into non flagellated amastigotes, which replicate and infect additional macrophages. Amastigotes ingested by sand-flies transform back into infective promastigotes. Depending on the host's innate and acquired immune status, systemic and visceral Leishmaniasis can be characterized by irregular fever, weight loss, enlargement of the spleen and liver, anaemia[2]. We present A 42 years-old man with long-lasting type 1 autoimmune hepatitis in immunosuppressive treatment. In January 2017 the patient started to experience low-grade unresponsive to empiric antibiotic therapy. The patient developed severe anemia and progressive multilineage cytopenia along with raised inflammation markers too. FDG-PET pointed out an increased glucose uptake in the liver, spleen, and the whole bone marrow. Consequently, a bone marrow biopsy was performed, with the evidence of Leishmania amastigotes inside macrophages, confirmed with serological positivity to anti-Leishmania antibody. Immunosuppressive

therapy was interrupted and treatment with amphotericin B was started at 4 mg/kg/die at day 1 to day 5, followed by single infusion at days 10, 17, 24, 31, 38. The bone marrow smear after treatment still evidenced few Leishmania amastigotes; unconventionally, two further doses of amphotericin B on days 45 and 52 were employed, in consideration of patient's immunosuppressed status, with infection resolution. In real-life, as represented by this case the additional administration of two doses of amphotericin B compared to the guidelines, offered an additional curative opportunity in patient in long-term immunosuppressive treatment.

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56ASM-0163 S6 | Adipose and hepatic aquaglyceroporins in energy metabolism: physiopathology and translational relevance

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Glycerol is an important intermediate in energy metabolism, being a key substrate for *de novo* synthesis of glucose (gluconeogenesis) during fasting and direct source of glycerol-3-phosphate for triglycerides synthesis (lipogenesis). Aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) are a group of aquaporin membrane channels that facilitate the movement of glycerol and some other neutral solutes in addition to water into or out of cells. Adipose tissue is a major source of glycerol released by adipocyte through AQP7 and AQP3. Through the bloodstream, lipolytic glycerol flows to the liver where it is imported by hepatocytes mostly by means of AQP9. The functional significance of AQP10 in fat tissue and liver is still debated. Although with distinctions between rodents and human, adipocyte and hepatocyte aquaglyceroporins are controlled by insulin and leptin via the PI3K/Akt/mTOR signaling cascade. Negative regulation is exerted by estrogens on fat and hepatic aquaglyceroporins likely explaining the sexual dimorphism that characterizes these AQPs in energy homeostasis. Adipose and liver AQP7 and AQP9 have been shown to play roles in energy balance disorders. AQP7 deficiency has been linked to abnormal triglycerides accumulation in fat tissue and adult onset of obesity and dysregulated expression of hepatic AQP9 is seen in animal models and patients with diabetes, obesity and/or fatty liver disease. Hepatocyte AQP9 is involved in the lipid-lowering activity exerted by the nutraceutical silybin through modulation of autophagy. Potent and isoform-specific blockers of AQP3, AQP7 and AQP9 are available and preclinical work is going on to investigate their relevance as drug targets in energy dyshomeostasis and other clinical disorders.

56ASM-0044 | Aquaporins and CFTR interaction: a possible channel for understanding male (in)fertility

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Background: Cystic fibrosis conductance regulator (CFTR) malfunction leads to dysregulation in male fertility. Aquaglyceroporins (AQPs) are essential channels to promote the transport of water and glycerol in cellular membranes. Sperm osmoadaptation is vital for the sperm journey throughout the male and female reproductive tract. Herein, we highlight a possible interaction between CFTR and AQPs on human sperm and their role in sperm membrane permeability.

Materials and Methods: Sperm samples from healthy men were used for measuring cellular membrane permeability assisted by AQPs. Permeability of human sperm cells was evaluated by stopped-flow light scattering after incubation with or without specific AQP3 (Z433927330), AQP7 (DFP00173), and CFTR (CFTRinh-172) inhibitors

and an unspecific AQP inhibitor (phloretin), for 10 minutes. Sperm cells were subjected to osmotic stress created by a hypertonic solution of glycerol (500 mM) to allow the study of AQPs permeability. Immunofluorescence was performed to study the localization of AQP3, AQP7, and CFTR on human sperm cells.

Results: Sperm glycerol permeability decreased after incubation with the AQP7 inhibitor but not when incubated with the AQP3 inhibitor when compared to the control group. CFTR inhibition also decreased glycerol membrane permeability in sperm. In fact, the decrease in permeability after AQP7 inhibition is positively correlated with the decrease in permeability after CFTR inhibition. CFTR is expressed in the equatorial section of the sperm head and diffusely throughout the midpiece. Whereas AQP7 is expressed throughout the head and midpiece of human sperm.

Conclusions: These results show that AQP7 is the AQP that most contributes to sperm cell glycerol permeability. Moreover, despite the CFTR is not able to transport glycerol, its inhibition also decreases glycerol permeability. CFTR and AQP7 are expressed in the same cellular localization in human sperm. That can highlight a potential functional and/or physical interaction between AQP7 and CFTR in human sperm with possible great impact in male (sub)fertility.

56ASM-0124 | Aquaporin-9 is involved in the systemic inflammation of LPS-induced endotoxic shock in mouse

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Background: Septic shock is the most severe complication of sepsis, characterized by a systemic inflammatory response, leading to multiple organ failure and dramatically high mortality (42% at 28 days after diagnosis). AQP9 is an aquaporin channel permeable to water and solutes such as glycerol and hydrogen peroxide, mainly expressed in hepatocytes and leukocytes. Recently, we showed involvement of AQP9 in maturation and inflammatory cytokines release from murine bone marrow dendritic cells under LPS challenging. Here, we investigate whether AQP9 plays a role in mouse systemic inflammation during endotoxic shock.

Materials and Methods: Wild type ($Aqp9^{+/+}$; WT) and *Aqp*9 gene knockout ($Aqp9^{-/-}$; KO) male mice were submitted to endotoxic shock by i.p. injection of LPS (40 mg/kg) and the related survival times were followed during 72 hours. Electronic paramagnetic resonance was employed to analyse the nitric oxide (NO) and superoxide anion (O_2^-) production. The expression of inducible NO-synthase (iNOS) and cyclooxygenase-2 (COX-2) in liver, kidneys, aorta, heart and lungs was evaluated by confocal microscopy after 6 hours of LPS challenging.

Results: LPS-treated KO mice survived significantly longer than WT mice, and 25% of the KO mice fully recovered from the endotoxin treatment. The LPS-injected KO mice showed lower inflammatory NO and O_2^- production and reduced iNOS and COX-2 levels through impaired NF-κB p65 expression/activation in liver, kidneys, aorta and heart compared to the LPS-treated WT mice. Treatment of a rodent hepatoma cell line with an AQP9 blocker (HTS13286) prevented the LPS-induced increase of inflammatory NO and O_2^- levels.

Conclusions: Altogether, these results suggest a role for AQP9, possibly at redox signaling level, in the early acute phase of LPS-induced endotoxic shock involving the NF-κB pathway. This may be of translational relevance in developing new therapeutic strategies for sepsis using clinically sustainable AQP9 blockers.

56ASM-0036 | Pathogenic role of gastric vascular barrier in *H. Pylori* and autoimmune gastritis

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Background and aims: The impairment of the gut vascular barrier, an anatomical structure capable of controlling the systemic dissemination of resident bacteria, has been shown to underlie inflammatory bowel disorders. Instead, nothing is known to date about the gastric vascular barrier, that may play a role in maintaining the local immunological homeostasis. We sought to evaluate the expression of plasmalemma vesicle-associated protein-1 (PV1), a marker of vascular barrier damage, in the stomach of healthy individuals (HC) compared to different types of gastritis, including autoimmune gastritis (AIG) and active *H. pylori* gastritis (HP).

Methods: We enrolled 5 HP patients (median age 54 years, 3 females) and sex- and age-matched patients with AIG ($n = 9$), as well as HC ($n = 9$). Perendoscopic

gastric corpus biopsies were collected. Total RNA was extracted from human biopsies by using the Direct-zol RNA Miniprep Kit (Zymo Research, Irvine, CA, USA). RNA was reverse transcribed with oligo(dT) and ImProm-II™ Reverse Transcriptase (Promega, Milan, Italy). cDNA expression was detected by Rotor-Gene Q 2Plex (Qiagen, Valencia, CA, USA). PV1 primers (genome wide bioinformatically validated primers sets) were provided by Qiagen (QuantiTect Primer Assays). Real-time PCR reactions were carried out using the Fast Sybr Green PCR kit (QuantiStudio 7 Flex R real Time PCR, Applied Biosystems, Thermo Fisher). Also, 3-millimeter-thick paraffin sections were used for immunohistochemistry by Dako Omnis automatic platform (Agilent, Santa Clara, CA, USA). An anti-plasmalemma vesicle-associated protein-1 (PV1)/PLVAP antibody (clone 174/2; LSBio, Seattle, WA, USA) was used to visualize the gastric vascular barrier.

Results: The immunohistochemical expression of PV1 resulted almost absent in the gastric corpus of patients with AIG, mildly expressed in HC, and over-expressed in HP. Also, significantly higher levels of PV1 transcripts were found in HP in comparison to both AIG and HP. PV1 transcript levels were reduced in AIG in comparison to HC, although the difference did not reach a statistical significance.

Conclusions: Compared to HC, gastric vascular barrier was disrupted in HP and appeared to be less permeable in AIG. It can be assumed that the impairment of the vascular barrier could play a pathogenic role in gastric diseases, just as in the gut. Larger studies are needed to confirm this assumption.

56ASM-0196 | Functional characterization of a Nav1.4 sodium channel mutation and a ClC-1 chloride channel mutation segregating together with myotonia in an italian kindred

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Background: Next-generation sequencing allowed identification of two mutations segregating in five relatives showing a myotonic phenotype: the novel p.K1302R mutation in SCN4A gene (hNav1.4 sodium channel) and the p.H838P mutation in *CLCN*1 gene (hClC-1 chloride channel). We performed a functional characterization of both mutations.

Materials and Methods: The mutations were introduced into the pRc/CMV plasmid containing the cDNA encoding wild-type (WT) human Nav1.4 and human ClC-1 channels. The sodium and chloride currents were recorded with whole-cell patch-clamp technique in HEK293 cells transfected with p.K1302R or p.H838P and compared to relative WT currents.

Results: Sodium currents generated by p.K1302R and WT hNav1.4 were very similar. Kinetics and voltage dependences of fast and slow inactivation were superimposed. The mutant channel showed a small negative shift (3 mV) in the voltage-dependence of activation, which increased the likelihood of the channel to open at more negative voltages. The p.H838P mutation caused a reduction in chloride current density and a small voltage-dependence shift towards less negative potentials, in agreement with the location of the mutation into the CBS2 domain of the C-terminus.

Conclusions: The results suggest that the mild functional alterations induced by p.K1302R and p.H838P may be asymptomatic when expressed alone. In individuals carrying both mutations, the combination of functional defects is likely responsible for the expression of the myotonic phenotype. (Supported by University of Bari – project Medineuropa).

56ASM-0087 | Clinical and functional analysis of a de novo mutation in the KV1.1 potassium channel in a patient with epilepsy developmental delay and ataxia

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Background: Kv1.1 channels, encoded by the *KCNA*1 gene, are localized in the central and peripheral

nervous system, where they regulate neuronal excitability. Pathogenic mutations in the *KCNA*1 gene are responsible for episodic ataxia type 1. Recently, *KCNA*1 variants have been associated with early onset developmental and epileptic encephalopathy (DEE) (D'Adamo et al., IJMS 2020). These latter variants are located in the channel pore, especially in the highly conserved Pro-Val-Pro (PVP) motif which is essential for Kv1.1 gating. We have identified a de novo mutation in *KCNA*1 in the PVP motif of Kv1.1 channel (P403A) in a girl affected by DEE and ataxia since birth. The patient has been treated with a combination of antiseizure medications with limited benefit. Finally, seizures and ataxia have been controlled with lacosamide and acetazolamide. The aim of this study was to provide a comprehensive description of the clinical case and to functionally characterize Kv1.1 mutant channel to define a genotype-phenotype-drug response correlation in *KCNA*1-related DEE.

Materials and Methods: To this aim, we transfected HEK 293 cells with Kv1.1 wild-type or P403A cDNAs and recorded potassium currents through whole-cell patch-clamp.

Results: P403A channels showed smaller potassium currents, voltage-dependent activation shifted by +25 mV towards positive potentials, slower kinetics of activation compared with Kv1.1 wild-type. Heteromeric Kv1.1+P403A channels, resembling the condition of the heterozygous patient, confirmed a loss-of-function defect.

Conclusions: The biophysical phenotype of P403A channels correlates with the clinical symptoms of the patient and supports the hypothesis that *KCNA*1 mutations associated with more severe DEE phenotype cluster in a highly conserved stretch of residues in the pore domain of Kv1.1 channel. There is no standardized therapy for *KCNA*1-related DEE and for this reason it is important to adopt a patient-centred approach to improve the disease outcome and the quality of life of patients.

56ASM-0079 | Calcium homeostasis and SOCE activity in impaired contractile function due to sarcopenia: effect of supplementation with BCAA-based formulation in aged mice

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Background: A key role of alterations in Ca^{2+} homeostasis and store-operated-calcium-entry (SOCE) process, has been claimed in sarcopenia and muscle weakness during aging (Brotto et.al., 2008). We recently demonstrated that branched-chain amino acids (BCAAs), which regulate metabolism and protein turnover, ameliorated muscle mass and function in a mouse model of atrophy (Mantuano et.al., 2020, 2021). On these bases, we investigated the mechanisms underlying Ca^{2+}-related alterations in aged muscle, meanwhile assessing the potential benefit of BCAAs supplementation.

Materials and Methods: 17-months-old male C57BL/6J mice received 12-weeks-treatment with BCAAs alone, or boosted with two equivalents of L-Alanine(2-Ala) or with dipeptide L-Alanyl-L-Alanine (Di-Ala), in drinking water. The outcome was evaluated on *in vivo*/*ex vivo* indices *vs* adult 6-months-old male C57BL/6J mice.

Results: *In vivo:* aged mice had a significantly lower torque of hindlimb plantar flexor muscles *vs* adult mice, which was partially recovered by BCAAs+2-Ala or Di-Ala (recovery scores: 27 and 60% respectively at 80 Hz). *Ex vivo:* aged soleus muscles showed a significant impairment in isometric twitch and tetanic force as well as in elastic properties. All mixtures improved these indices (recovery scores between 25 and 60%). Ca^{2+} imaging confirmed SOCE decrease and Ca^{2+} concentration increase in aged *vs* adult mice without alteration of STIM1/Orai1/TRPC1 mRNA levels, known actors of SOCE mechanism. Interestingly, gene expression analysis showed a significant reduction of Mitsugumin29 (MG29), SERCA1; Ryr1, and an increase of Mitsugumin53 (MG53) in aged *vs* adult mice. All these alterations were restored by BCAAs+2-Ala treatments.

Conclusions: In aging-related sarcopenia, Ca^{2+} homeostasis and SOCE alterations may result from signaling dysfunction downstream SOCE components and pointing towards MG29, a muscle synaptophysin-related protein whose alterations appear to be related to dysfunctional SOCE in neonatal myofibers (Brotto et.al.,2008) and MG53, a TRIM-family protein involved in cell membrane repair (Ahn et.al., 2016). BCAAs on muscle biomarkers under examination highlight their interest in counteracting the sarcopenia-related alterations.

56ASM-0084 | LKB1 as a new player in metabolic and epigenetic dysregulation in dystrophic myofibers

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Background: Histone deacetylases (HDACs) play a role in Duchenne muscular dystrophy. Dystrophic myofibers display a failure of HDACs inactivation, which increase their epigenetic silencers activity, suppressing the expression of important myogenic and metabolic factors. In detail, HDACII, when phosphorylated, shuttle from nucleus to cytoplasm, where they are inactive. We focused on the potential defect in the HDACII phosphorylating pathways promoted by the adenosine monophosphate-activated protein kinase (AMPK). An upstream activator of AMPK is the liver kinase B1 (LKB1) which forms a heterotrimeric complex with two accessory proteins, the pseudokinase STE20-related adaptor and the scaffolding mouse protein 25. In this frame, we investigated whether a dysfunction of LKB1 plays a role in the altered mechano-metabolic coupling of dystrophic myofibers.
Materials and Methods: Thus, we performed gene and protein expression analyses in gastrocnemius muscles of two different dystrophic murine models, the C57BL/10 *mdx* and the novel DBA/2J *mdx* mouse, at adult age. In addition, we assessed the extent of the HDACII pathway defect in 6-month-old exercised *mdx* mice, a condition wherein AMPK should be physiologically activated.
Results: Our data showed, for the first time, by Western Blot analysis, a reduction of the heterotrimeric complex levels in *mdx* (-57,9%) and D2 *mdx* (-65,8%) mice vs. their respective WT controls, confirmed at gene level for LKB1 (-52,8% and -48,2%). This evidence might explain the reduced phosphorylation of HDACII observed and the diminished expression of myocyte enhancer factor-2c gene, the main target silenced by HDACII. Notably, we highlighted a further impairment of the heterotrimeric complex integrity in exercised *mdx* mice, despite the significant increase of pHDACII/HDACII ratio detected. This latter might be related to the activation of alternative phosphorylation pathways, likely controlled by stress-related ROS production or calcium and currently under investigation.
Conclusions: Our study suggested LKB1 as novel possible players in dystrophic progression, paving the way for future preclinical studies.

56ASM-0042 | Topology of sodium-dependent phosphate transporter NaPi2b in live ovarian cancer cells

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Background: The sodium-dependent phosphate transporter NaPi2b that belongs to the SLC34 family of transporters which mainly responsible for phosphate homeostasis in the humans. NaPi2b is overexpressed in several malignancies, making it attractive target for cancer therapy. Although topology of NaPi2b was predicted *in silico*, no direct experimental data for orientation of NaPi2b extracellular domains are available. Study of the localization of individual domains of the transporter will reveal new promising targets for the development of therapeutic monoclonal antibodies
Materials and Methods: The CCTOP server was used to predict the number of transmembrane domains in NaPi2b and the topology of its domains. Fluorescent confocal microscopy with mouse polyclonal antibodies against extracellular domain (ECD) of NaPi2b, monoclonal antibodies against N-terminal domain of NaPi2b, and rabbit monoclonal antibodies against C-terminal domain of NaPi2b (Cell Signaling, USA) were used to determine localization of these domains in live or fixed and permeabilized ovarian cancer line OVCAR-4 cells.
Results: According to CCTOP programs the number of predicted transmembrane (TM) domains ranges from eight to eleven *membrane-spanning regions* with N-terminal, ECD, and C-terminal domains potentially facing either the cytoplasm or outside. We determined the orientation of all three domains of NaPi2b in OVCAR-4 cells, showing that N- and C-terminal domains are located intracellularly, therein ECD is located extracellularly. The green, fluorescent signal, which is seen in Z-stacking, verifies the membrane residence of NaPi2b.
Conclusions: Our results supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and Russian Science Foundation (RSF) project № 20-14-00166 provide the first experimental evidence for the intracellular location of the N- and C-termini, and the extracellular location of the largest extracellular domain (loop) of untagged NaPi2b transporter

localized primarily to the plasma membrane as demonstrated by confocal microscopy.

56ASM-0030 | Developmental profile of the flurothyl-induced epileptiform discharges in the rat somatosensory cortex

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Background: Flurothyl is a volatile agent which readily induces generalized tonic-clonic paroxysmal cortical discharges and which is widely used in epilepsy research. However, developmental aspects of the flurothyl-induced epileptic activity in cerebral cortex remain poorly understood.
Materials and Methods: We explored age-dependence of the flurothyl-induced epileptiform activity using multisite silicone probe recordings of local field potentials (FPs) and multiple unit activity (MUA) from cortical barrel columns of urethane-anaesthetized head restrained rats aged from postnatal days (P) 6 to 365.
Results: Flurothyl inhalation induced electrographic seizures which duration varied from 20 to 300 seconds but did not show any age-dependence. Epileptiform activity was organized in bursts of population spikes (PS) which displayed similar current-source density depth-profile across all ages with the leading sinks and MUA in the infragranular layers followed by activation of the superficial layers. However, PSs in the neonatal and juvenile animals were of smaller amplitude, longer duration and occurred at lower frequency. During the period from P6 to P23, the PSs' frequency linearly increased along with a progressive increase in the PSs' amplitude and shortening in PSs' duration. The power spectra of the flurothyl-evoked seizures also showed a gradual increase in the power and its shift towards higher frequency values with age. By P25 the main electrographic parameters of the flurothyl-evoked epileptic discharges attained steady state levels.
Conclusions: The study revealed a significant age dependence of the main electrographic characteristics of the flurothyl-induced electrical epileptic activity in the rat barrel cortex during the first postnatal month. These developmental changes in epileptic activity likely involve maturation of intrinsic neuronal firing properties and development of intracortical connectivity.
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56ASM-0037 | The role of thymic stromal lymphopoietin pathway in human autoimmune gastritis

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Background and Aims: Autoimmune gastritis (AIG) is an immune-mediated disorder affecting the oxyntic mucosa, leading to progressive mucosal atrophy. The pathogenic mechanisms underlying AIG are complex and yet to be clearly defined. Previous experimental models suggested a potential role of thymic stromal lymphopoietin (TSLP), an epithelium-derived anti-inflammatory cytokine involved in T cell maturation, and its receptor (TSLPR) in counteracting the inflammatory process. We therefore conducted an *ex vivo* study to assess the TSLP pathway role in human AIG.
Methods: Eighteen AIG patients (median age 59 years, 12 females), and 10 age and sex-matched healthy controls (HC) and H. pylori-infected patients (HP) were enrolled. Perendoscopic gastric corpus biopsy specimens were placed on iron grids in the central well of an organ culture dish and placed in a tight chamber with 95% O2/5% CO2 at 37°C. Biopsies were cultured for 24 hours in serum-free HL-1 medium (Cambrex Bio Science, Milan, Italy), added with antibiotics, with or without 10 ng/mL short TSLP. After 24-hour culture, supernatants and tissues were used to assess the cytokine production in AIG. Total RNA was extracted from biopsies by using the Direct-zol RNA Miniprep Kit (Zymo Research, Irvine, CA, USA). TSLPR and IL-7R primers were provided by Qiagen (QuantiTect Primer Assays). Finally, 5-μm-cryostat sections were fixed

in cold acetone or in paraformaldehyde 4%. Primary antibodies were incubated overnight at 4°C, namely anti-total TSLP (#ab47943; Abcam) and anti-TSLPR (#743961; BD Biosciences). Slides were visualized under a Leica TCS SP8 laser scanning confocal microscope.

Results: Short TSLP significantly reduced TNF-α, while it had no significant effect on the other tested cytokines. No difference was found among the three groups with regards to TSLPR levels, while IL-7R was significantly more expressed in AIG compared to HC and HP. At immunofluorescence, both IL-7R and TSLPR were more expressed in AIG compared to HC and HP. Total TSLP expression, assessed by immunofluorescence, was also more evident in AIG compared to HC and HP. Short TSLP transcripts were significantly increased in AIG compared to HC.

Conclusions: In this *ex vivo* study, TSLP and TSLPR levels appear to be significantly higher in AIG than in control groups, suggesting a pathogenic role for this pathway. It can be assumed that the lamina propria over-expression of TSLP could function as a compensatory -although ineffective- anti-inflammatory mechanism.

56ASM-0038 | Role of NAMPT as a pro-inflammatory cytokine in untreated celiac disease

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Background: The nicotinamide phosphoribosyltransferase (NAMPT), characterized by a pro-inflammatory and cytokine-like activity, is overexpressed and oversecreted in inflammatory bowel disease (IBD), a group of complex, immune-mediated chronic conditions, marked by prolonged inflammation of the gastrointestinal tract, whose incidence and prevalence are increasing worldwide. A link between enhanced levels of NAMPT and worse prognosis has been reported. However, less is known about the role of NAMPT in the inflammatory pathway of other gastrointestinal disorders, such as celiac disease (CD). We aimed to explore the inflammatory potential of NAMPT in intestinal organ cultures from patients with celiac disease (CD), an autoimmune disorder that occurs in genetically predisposed individuals.

Methods: To study the expression of NAMPT in CD organ cultures, we collected biopsies with Jumbo forceps during upper GI endoscopy from healthy subjects, as well as from CD patients. Biopsies from CD patients (with treated and untreated CD) ($n = 10$) were processed for RT-qPCR to assess the mRNA expression of NAMPT compared to healthy controls ($n = 10$). A Student's t test was used for comparing the mRNA expression among groups.

Results: We found a statistically significant increase in NAMPT mRNA expression in patients with untreated CD compared to both treated CD and healthy controls. Remarkably, patients with treated CD displayed roughly the same NAMPT mRNA expression of healthy controls (p not significant).

Conclusion: These data indicate that NAMPT may act as a pro-inflammatory cytokine in autoimmune pathologies of the gastrointestinal tract and trace a new way to investigate as a potential therapeutic target and biomarker in gastrointestinal disorders, such as CD.

56ASM-0047 | Analysis of the sodium-dependent phosphate transporter NaPi2b expression in human tumor cell lines

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Background: Sodium-dependent phosphate transporter NaPi2b is a membrane glycoprotein essential for phosphate homeostasis. Higher levels of NaPi2b expression were detected in various malignancies including ovarian, breast and lung cancers. Thus, NaPi2b is a potential target for anticancer treatment based on monoclonal antibodies. The search for convenient in vitro models is still relevant for the investigation of NaPi2b structure in tumor cells and new NaPi2b specific drugs development. The purpose of the study was to evaluate the protein expression level in human tumor cell lines, namely pancreatic cancer AsPC-1; BxPC-3; MIA PaCa-2; Capan-2; CFPAC-1; PANC-1; HPAF-II, lung cancer H441; H460; A549, ovarian cancer Caov-3; SKOV-3; A1847; OVCAR-3; OVCAR-8; OVCAR-4, and breast cancer MDA-MB-436; T-47D, MDA-MB-231.

Materials and Methods: The NaPi2b expression level was detected by Western Blotting with the primary mouse monoclonal antibody targeting NaPi2b N-terminal domain and secondary HRP-conjugated antibodies. Chemiluminescent signal was detected by gel imaging system ChemiDoc XRS+ (Bio-Rad, USA). Quantification

of the signal intensity was conducted using Image Studio Lite software (LI-COR Biosciences, USA) and normalized by GAPDH loading control.

Results: The protein expression of NaPi2b transporter was heterogeneous in the cell lines and varied from 0 to 4.5 relative units. NaPi2b expression was detected in ovarian cancer OVCAR-3; OVCAR-4 and lung cancer H441 and A549 cell lines. For the first time NaPi2b expression was revealed in pancreatic cancer namely pancreatic cell lines CFPAC-1 and PANC-1. All the other cell lines showed no expression of NaPi2b.

Conclusions: The results of work supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and Russian Science Foundation project №20-14-00166 show that cell lines expressing NaPi2b, namely OVCAR-3; OVCAR-4; H441; A549; CFPAC-1 and PANC-1 can be used for structural and functional research on the endogenous NaPi2b, whilst others without NaPi2b expression might be involved in structural and immunogenic features of recombinant NaPi2b including mutant variants.

56ASM-0049 | Zinc l-carnosine exerts an anti-inflammatory effect in the gastric mucosa of patients with autoimmune atrophic gastritis

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Background and Aims: Autoimmune atrophic gastritis (AAG) is a progressive, chronic, inflammatory disease of the stomach, involving the body and the fundus mucosa. The inflammation leads to the destruction of parietal cells, resulting in gradual hypo-achlorhydria and vitamin B12 deficiency. To date, no therapy proved effective in healing or improving mucosal inflammation and atrophy in AAG. Zinc l-carnosine is a chelate compound of zinc and l-carnosine, which has shown anti-inflammatory and anti-apoptotic properties in several conditions, and it is currently being used for treating peptic ulcer disease. We here report the *ex vivo* effect of zinc I-carnosine on histological gastric samples from patient with autoimmune atrophic gastritis.

Methods: Gastric corpus biopsy samples were obtained through endoscopy from 18 AAG patients (median age 59 years, 6 male and 12 females; all with severe gastric atrophy). Biopsies were located on iron grids within the central well of a culture dish. Dishes were placed in a tight chamber at 37°C, with 95% O2 and 5% CO2. Biopsies culture occurred for 24 hours in serum-free HL-1 medium (Cambrex Bio Science, Milan, Italy), added with antibiotics, with or without 10 ng/mL zinc l-carnosine. After 24-hour culture, the concentrations of TNF-α, IL-15; IFN-γ, IL-17; IL-6, and IL-21 were measured in organ culture supernatants using the Luminex x-MAP technology (Luminex Corporation, DiaSorin Company, Austin, TX, USA).

Results: The concentrations of the tested cytokines (pg/mL) in the supernatants of organ culture gastric corpus biopsies are reduced when zinc l-carnosine is added. The downregulation was statistically significant for IFN-ɣ, TNF-α, IL-21; IL-6, and IL-15, but not for IL-17.

Conclusions: Zinc l-carnosine exerts an anti-inflammatory effect in the gastric corpus mucosa of patients with AAG, in an *ex vivo* experiment. This may potentially improve the gastric histopathological lesions or could block their evolution. *In vivo*, prospective, studies are needed to corroborate our results.

56ASM-0053 | The role of gabaergic transmission during epileptiform activity in the adult rat brain in vivo

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Background: Epilepsy is one of the most common diseases of the nervous system, characterized by repeated paroxysmal neuronal activity, caused by increased hypersynchronous activity in the cortical network. In vitro studies demonstrated an increase in the intracellular concentration of chloride during epileptiform activity that could result in the GABA polarity function change from inhibitory to excitatory. But the question remains whether GABA changes its polarity during the epileptiform activity in vivo.

Materials and Methods: To answer this question, we characterized the intracellular chloride concentration during the epileptiform activity using single channels and local field potential recordings in adult Wistar rats in vivo. Using the Goldman-Hodgkin-Katz equation, the resting membrane potential of the cell and the reversal potential for GABA were calculated in control and in conditions of epileptiform activity evoked by 4-aminopyridine (100 mM).

Results: Our preliminary results showed that during epileptiform activity, the neuronal resting membrane potential was strongly depolarized (-27.73 ± -7.94 mV, while in control -53.35 ± -9.29 mV). The depolarizing shift was also observed for GABA (reversal potential shifted from

-60.20 ± -10.33 mV in control to -32.68 ± -0.29 mV during the epileptiform activity).

Conclusions: In spite of GABA reversal potential shift, it was more negative compared to resting membrane potential, meaning that GABA served as the hyperpolarizing and inhibitory neurotransmitter during the epileptiform activity in vivo. Further research is required to increase the patched cells number and the mechanisms underlying the intracellular chloride concentration increase.

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56ASM-0054 | Reinforcement of excitatory input at the single cell level during evoked focal epileptic activity in the adult rat somatosensory cortex

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Background: Epilepsy is a chronic disease of the nervous system, linked to the hypersynchronization of the neuronal activity. One of the main mechanisms is the impairment of the excitation/inhibition balance due to the excitation predominance, that is clearly shown in vitro studies. But it is still unknown whether an increase in excitation also characterizes the epileptiform activity in vivo.

Materials and Methods: To answer this question, we characterized the frequency of excitatory postsynaptic currents in control and during evoked epileptic activity. The experiments were done on maturing rats p18-27. Single whole cell recordings in vivo were done (at membrane potential fixed at -60 mV) in control and during epileptic activity evoked by local injection of 4-aminopyridine (100 mM, 0.5-1 μl).

Results: Our results showed that epileptiform activity was associated with increased excitation in the neural network. Comparison of glutamate currents frequency showed an increase during epileptic activity by 4 times (from 1.1 ± 0.12/sec in control to 4.32 ± 0.47/sec). We have equally observed that during epileptic activity, there is an increase in synchronicity of the excitatory postsynaptic currents (in control the modulation index was 0.52 ± 0.03 and during epileptiform activity 0.67 ± 0.04).

Conclusions: Although further studies are required, our preliminary data indicate an increase in the excitatory inputs and their synchronization during the epileptiform activity in vivo. It could be assumed that epileptic activity led to a balance shift towards excitation, being in agreement with data published from in vitro studies.

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56ASM-0055 | Modulation of the immature hippocampal activity in vivo by dexmedetomidine

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Background: In vivo studies often require the use of anesthesia. One of the most used injectable anesthetics is urethane. However, urethane use is limited due to its side effects, which results in its restrictions in vivo studies. That put a question of urethane replacement by another anesthetic. In order to find the possible candidate to replace the urethane in neuropsychological studies in vivo, we have tested the α-adrenergic receptor agonist dexmedetomidine hydrochloride (DexDomitor). While its dose-dependent sedative and anesthetic efficiency is well described, the question remains about its effects on cortical activity.

Materials and Methods: To answer this question, we have done extracellular recordings of the hippocampal activity in the neonatal rats in vivo. The effects of DexDomitor and urethane were compared by characterization of the changes in the immature pattern of hippocampal activity – early sharp waves (eSPW).

Results: Both anesthetics strongly suppresses the eSPWs frequency (DexDomitor for 60% and Urethane for 83%). This was accompanied by a decrease in motor activity by 1.5 and 3 times, respectively. eSPW duration and amplitude remained almost unchanged (but a drop in the amplitude of the eSPW by 25% in the stratum oriens was observed after urethane).

Conclusions: We demonstrated that in spite of strong sedative and anesthetic effects, the α-adrenergic receptor agonist dexmedetomidine hydrochloride weakly modified the immature hippocampal activity. Despite the necessity to continue further investigations to characterize the dose-dependence, DexDomitor could be considered to be the candidate for urethane replacement in in vivo neurophysiological studies.

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56ASM-0056 | Lipid-assisted folding defines recognition of conformationally exposed cancer specific napi2b epitope by monoclonal antibodies in ovarian cancer cells

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Background: Large extracellular extramembrane domain (ECD) of the sodium-dependent phosphate transporter NaPi2b, overexpressed on the cell surface of a number of tumors comprise a conformational epitope is recognized by therapeutic monoclonal antibodies. Since membrane proteins folding depends on a given lipid profile we postulate that NaPi2b ECD folding is structurally supported by dynamic process driven by interplay of individual lipids which in turn contribute to epitope recognition.
Materials and Methods: Eastern-Western blot analysis of ovarian cancer cells OVCAR-4 was applied to determine the ECD epitope recognition using in-house conformation specific and commercial monoclonal antibodies (Cell Signaling, USA) in presence of the following phospholipids: DOPC, DOPS, POPE and DOPE. Identification of the lipid binding motifs was carried out by searching for conserved unique contiguous amino acid patterns within multiply aligned sequences of proteins of the SLC34 family using Muscle software and known lipid-binding signatures as query sequences.
Results: Influence of the phospholipids on the signal intensity is decreased in the row of DOPE > POPE > DOPS≫DOPC preblotted to PVDF membrane support (Eastern blotting) prior Western blotting and immunostaining with in-house conformation sensitive antibodies. The affinity and selectivity of aminophospholipid-ECD interactions is ensured by presence of putative PS/PE binding motif, which was identified bioinformatically within the region of ECD proximal to membrane interface.
Conclusions: According to results supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and Russian Science Foundation project No 20-14-00166 aminophospholipids can contribute to the ECD foldability which ensures the availability of the epitope for the monoclonal antibodies. The formation of the potential cancer-specific epitope can be influenced by changes of ECD conformation due to its interaction with the plasma membrane promoted by the putative PS/PE binding motif. The results contribute to understanding the dynamic structural and functional features of membrane proteins presenting an excellent targets for specific therapeutic antibodies in cancer cells.

56ASM-0072 | Acute stress selectively reduce efficiency of perisomatic inhibition at synapses formed by hippocampal CCK/CB1-positive basket interneurons

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Background: The proposed involvement of a subpopulation of CCK-positive perisomatic interneurons in the formation of the post-stress response follows from in vivo experiments demonstrating stress-induced changes in the theta rhythm of the hippocampus. Functioning of CCK-positive synapses is controlled by the endocannabinoid signaling system through activation of presynaptic CB1 receptors. Numerous studies show changes in endocannabinoid tone and expression levels of cannabinoid receptors in response to various types of stress. In general, the current literature lacks data on the effect of acute stress on the components of endocannabinoid cascade, which includes endocannabinoid metabolism and CB1 receptor functioning.
Materials and Methods: The acute stress in the wild type mice at the age P28-42 was induced by immobilizing the animal for 60 min. Immediately after stress induction, the animal's brain was removed and sliced for subsequent in vitro experiments. In the control group, the brain was removed a maximum of 10 min after removal from the home cage. All experiments were done by using patch-clamp paired recordings.
Results: Paired patch-clamp recording of connected presynaptic CCK-positive interneurons and postsynaptic CA1 pyramidal cells reviled that acute short-term stress causes significant suppression of inhibition in these synapses.
The amplitude of postsynaptic GABAergic responses was significantly lower in slices of stressed mice. The application of the CB1 receptor antagonist AM 251 led to the restoration of the amplitude of postsynaptic responses to values characteristic for unstressed animals, which indicates the involvement of CCK/CB1 positive perisomatic cells in the formation of an early stress response.
Conclusions: In the hippocampus, acute stress causes an increase in endocannabinoid synthesis, which, in turn,

suppresses the release of GABA from the terminals of CCK/CB1-positive hippocampal neurons.

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56ASM-0082 | Growth hormone secretagogues exert anti-fibrotic actions in duchenne muscular dystrophy: a preclinical study

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Background: Growth hormone secretagogues (GHSs) activate GHS-R1a, control inflammation and metabolism, enhance GH/IGF-1 mediated myogenesis, and inhibit angiotensin-converting enzyme (ACE), all pathways of interest for reducing fibrosis in Duchenne muscular dystrophy (DMD). Our study aims to provide preclinical evidence for potential benefits of GHSs in DMD, via a multidisciplinary *in vivo* and *ex vivo* comparison of two *ad hoc* synthesized compounds with wide but different profile (EP80317 and JMV2894) in *mdx* mice, paralleled by *in vitro* assays on wild type (wt, H2K-2B4) and dystrophic (H2K-SF1) cell-lines.

Materials and Methods: Four-week-old *mdx* mice were treated for 8 weeks (T0-T8) with EP80317 or JMV2894 (320 μg/kg/d, s.c.). Wt and dystrophic myoblasts were treated with EP80317 or JMV2894 at increasing concentrations and analyzed at 12, 24, 48-hour time points.

Results: *In vivo*, both GHSs increased mice forelimb force (recovery score vs wt value, RS: 20% for EP80317 and 32% for JMV2894 at T8), and reduced diaphragm (DIA) ultrasound echodensity (RS: 69% and 75%, respectively), whilst only EP80317 improved DIA amplitude (RS: 110%). *Ex vivo*, both drugs ameliorated DIA isometric contraction (*e.g.* RS: 40% for tetanic force), with EP80317 also partially preserving *mdx* DIA response to eccentric stimuli. Both drugs, in particular JMV2894, reduced collagen (assessed by Masson trichrome), in gastrocnemius muscle and mostly in DIA. Gene expression is ongoing.

Drug cytotoxicity was excluded by *in vitro* assays, on both 2B4 and SF1 cell-lines. Preliminary qRT-PCR experiments showed a reduced SF1 commitment to myogenesis as revealed by a reduced time-dependent increase of Pax7; MyoD, myogenin and desmin levels. JMV2894 was the most effective in modulating the expression of these markers in differentiating SF1. Moreover, both drugs inhibited ACE activity.

Conclusions: We confirmed the multiple actions of GHSs in dystrophic settings, disclosing an anti-fibrotic action of therapeutic interest. [Supported by AFM-Téléthon #22199].

56ASM-0090 | Effect of acute stress on SPW-R driven feedforward inhibition of layer 5 pyramidal cells in entorhinal cortex

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Background: It is known that sharp wave ripple oscillations (SPW-R) are conducted into the deep layers of the entorhinal cortex through direct hippocampal projections. The primary targets of the hippocampal inputs are layer 5 pyramidal cells and interneurons, in particular, cortical CCK/CB1-positive interneurons that innervate 5A and 5B pyramidal neurons, controlling efficiency of SPW-R propagation. Considering that stress can increase endocannabinoid tone in various brain regions, decreasing the influence of CCK/CB+ interneurons on network activity, it's important to understand whether it has a modulating effect on the functioning of the hippocampal-entorhinal loop.

Materials and Methods: In submerged acute slices from stressed and naïve mice (P28-42) we used simultaneous recording of hippocampal SPW-R oscillations combined with patch-clamp recording from identified neurons.

Results: Analysis of the involvement of cortical CCK-positive interneurons in the conduction of SPW-R driven feedforward inhibition showed:

1)The probability of firing APs during SPW-R in EC CCK/CB1+ interneurons significantly higher than that in PV neurons.

2)The main component of feedforward inhibition during SPW-R pyramidal neurons is the release of GABA from CB1 expressing terminals.

3)In slices obtained from the brains of stressed mice, the contribution of CCK/CB1+ interneurons to the inhibitory response induced by SPW-R in layer 5 pyramidal neurons of the EC was significantly reduced. However, blockade

of CB1 receptors led to an increase in IPSP amplitudes up to the values observed in sections of the control group of mice.

Conclusions: We showed that cortical CCK/CB1-positive interneurons play the major role in hippocampus driven feedforward inhibition of deep layers of EC. However, the influence of inhibition provided can be significantly reduced under acute stress due to the activation of presynaptic CB1 receptors.

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56ASM-0092 | Gender specificity of early postnatal stress influence on long-term synaptic plasticity at hippocampal excitatory synapses

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Background: Several studies have shown that in adult animals exposed to early postnatal stress, long-term potentiation (LTP) is selectively suppressed in the dorsal and enhanced in the ventral hippocampus. It is known that LTP in the CA1 apical dendrites of pyramidal neurons reaches higher values than in the basal ones, which may be associated with increased selective expression of GluA1 homomeric AMPA channels. Therefore, we studied the effect of early postnatal stress on the LTP level in apical and basal synapses, also analyzed possible gender characteristics of the response to stress.

Materials and Methods: Chronic stress was induced by weaning from the mother for 3 hours from the 2nd to the 10th postnatal day. Analysis of the impact of stress was carried out at the age of 28 to days and for control unstressed mice, respectively. LTP levels were recorded and analyzed using the patch-clamp recording technique in voltage clamp mode on CA1 pyramidal neurons in acute slices..

Results: We found that in naive females, the levels of LTP at synapses both on the apical (340%) and basal dendrites (231%) were significantly higher than the levels observed in unstressed males (apical synapses: 220%; basal synapses: 163%). In males, early postnatal stress did not have significant effect on the level of potentiation (apical synapses: 221%; basal synapses: 161%). However, in females early postnatal stress caused significant reduction of LTP levels at synapse on the apical (144%) and basal dendrites (127%).

Conclusions: Early postnatal stress selectively reduces the ability to generate LTP at excitatory synapses on CA1 pyramidal cells in female. In males, early postnatal stress did not cause statistically significant changes in the level of potentiation.

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56ASM-0098 | Tamoxifen reduces hepatocyte growth factor secretion in breast cancer cells with high NLRP3 expression

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Background: Tamoxifen is widely used for breast cancer chemotherapy. However, in some cases, the resistance to tamoxifen could be explained by inflammation. One of the central mechanisms of inflammation is mediated by Nod-like receptor protein 3 (NLRP3). NLRP3 was shown to promote proliferation, survival, metastasis, angiogenesis, and immunosuppression of breast cancer cells. Recently, the hepatocyte growth factor (HGF), a pleiotropic cytokine, was shown to protect from the damage caused by inflammation. However, the effect of NLRP3 mediated tamoxifen resistance on HGF secretion by breast cancer cells remains unknown.

Materials and Methods: this study, we investigated the association between NLRP3 expression and HGF secretion by the tamoxifen-treated breast cancer cell lines with different pathological features. MCF7, a breast cancer cell line expressing the estrogen, progesterone and glucocorticoid receptors as well as the triple negative MDA-MB231 cell line representing the more aggressive type of breast cancer compared to MCF7.

MCF7 and MDA-MB-231 were treated with lipopolysaccharide and ATP to induce NLRP3 protein expression. Then, each cell lines were treated with tamoxifen. NLRP3 activation was analyzed by western blot. The effect of tamoxifen on HGF secretion was determined using Bio-Plex

Pro Human Chemokine Panel, 40-Plex. Statistical analysis was performed by one-way ANOVA with Tukey's analyses.

Results: We found that NLRP3 protein expression was higher in MDA-MB-231 as compared to MCF7 cells. While tamoxifen treatment increased the HGF release in MCF7 cells ($p = 0.002$), it did not affect HGF secretion in MDA-MB-231 cells.

Conclusions: Our data showed that one of the mechanisms of tamoxifen attenuation of inflammation is via HGF secretion. It appears that NLRP3 overexpression in triple-negative breast cancer cells abolishes this protective effect of tamoxifen, which suggests the requirement of NLRP3 inhibitors into the therapeutic regimens of tamoxifen therapy to replace the biological activity of HGF in these tumors.

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56ASM-0143 | Probing polarity of GABA-activated chloride permeable ionic channels using cell-attached current-clamp recordings

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Background: Cell-attach current-clamp (CA/CC) recordings have been suggested as an approach to assess the polarity of GABA(A)-receptor mediated postsynaptic responses. However, the accuracy of this approach has not been yet verified.

Materials and Methods: We used concomitant dual cell-attached and whole-cell current-clamp recordings from the soma of cortical L5 neurons of somatosensory cortex and CA3 pyramidal cells of neonatal hippocampus in brain slices in vitro. Synaptic GABA(A)-receptor mediated responses were evoked by electrical stimulation in the presence of the ionotropic glutamate receptor antagonists CNQX and d-APV.

Results: During whole-cell (WC) recordings, amplitude and polarity of the GABA(A)-receptor mediated postsynaptic potentials (GABA-PSPs) depended on the chloride concentration in the patch pipette solution and on the holding membrane potential. Concomitant CA/CC recordings from the same neurons revealed GABA-PSPs, which polarity coincided with WC GABA-PSPs. The amplitude and polarity of GABA-PSPs in CA/CC dependence on the membrane potential and intracellular chloride concentration matched that of GABA-PSPs in WC/CC. However, the kinetics of GABA-PSPs in CA/CC was slower and the amplitude of CA/CC GABA-PSPs was nearly half of GABA-PSPs in WC/CC. Action potentials evoked by depolarizing GABA-PSPs, or arising at rebound of hyperpolarizing GABA-PSPs at depolarized membrane potentials were even stronger attenuated in amplitude and slowed down than synaptic potentials in CA/CC configuration. CA/CC recordings also revealed depolarizing and often excitatory GABA-PSPs in the majority of neonatal hippocampal neurons.

Conclusions: CA/CC recordings enable reliable assessment of the GABA-PSP polarity and excitatory/inhibitory GABA actions.

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56ASM-0146 | The adjuvant property of PAMAM when immunizing with plasmid coding for puumala virus glycoproteins

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Background: Hemorrhagic fever with renal syndrome (HFRS) is an emerging infectious disease that is endemic in multiple countries. Most cases of HFRS are reported in the Volga region of Russia, which identifies the Puumala virus "PUUV" as a pathogen. Severe consequences of HFRS and prevalence among the young calls for the development of preventative measures against this disease. Our goal was to analyze the efficacy of polyamidoamine (PAMAM) dendrimers transferring plasmid construction pBud-PuuM-EGFP. We demonstrated an activation of humoral response after plasmid administration *in vivo*.

Materials and Methods: Female C57Bl/6 mice received intramuscular injection of plasmid construction pBud-PuuM-EGFP encapsulated in PAMAM. Humoral immune response after in vivo administration of plasmid was analyzed by ELISA.

Results: Administration of pBud-PUUM-EGFP with PAMAM resulted in significant augmentation of anti-orthohantavirus IgG after 14 days ($p = 0.0235$) and 28 days ($p < 0.0001$). This data indicates an efficient transgene

delivery using PAMAM as a carrier for orthohantavirus plasmid constructions.

Conclusions: In summary, our data provides evidence that PAMAM with plasmid construction pBud-PUUM-EGFP can induce a specific humoral immune response. Obtained data indicate PAMAM could become an efficient vehicle for delivery plasmid constructions for vaccination.

56ASM-0147 | Prolonged immune response induced by sputnik V vaccine

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Background: SputnikV is a vaccine against SARS-CoV-2 developed by the Gamaleya National Research Centre for Epidemiology and Microbiology (GRCEM). Recent studies showed that both humoral and cellular responses were induced by the vaccine, but the mechanisms remain largely unknown.

Materials and Methods: Serum samples were collected from 40 vaccinated people and 40 convalescent COVID-19 patients. Anti-SARS-CoV-2 antibodies were measured using the Coronapass ELISA test. Additionally, we analyzed T cell immune response by using a Tigra test. Statistical analysis was performed in the R environment. Statistically significant differences between comparison groups were accepted as $p < 0.05$, assessed by the Kruskal-Wallis test with Benjamini-Hochberg adjustment for multiple comparisons.

Results: The first dose of SputnikV vaccine elicited an antibody response, while the second dose enhanced the antibody production. However, the second dose increased the antibody response significantly higher as compared to the first dose. We also observed anti-SARS-CoV-2 antibodies in samples collected on day 90 after the first dose of vaccine. Next, we have found that the antibody response in convalescent COVID-19 was higher than that in vaccinated with SputnikV on day 21; however, it was lower than that on day 42 after the immunization.

An increased number of IFN-gamma spots were detected in a Tigra test from 26 immunized individuals at day 90 as compared to 12 anti-SARS-CoV-2 antibody-negative controls. Our data provide strong evidence for the ability of SputnikV vaccine to induce a T cell immune response.

Conclusions: We have identified similarities between an immunization-induced response and that developed after the recovery from SARS-CoV-2 infection. Our finding of reactivity was still evident 90 days after vaccinating, demonstrating that the vaccine has long-term activation and successful generation of both humoral and cellular immunological memory which can explain the efficacy of the SputnikV vaccine against SARS-CoV-2 infection.

56ASM-0153 | Ion channels and duchenne muscular dystrophy: electrophysiological asset of wild-type and dystrophic myocytes during myogenesis

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Background: In skeletal muscle, several ion channels interact with the dystrophin complex, which is disrupted in Duchenne Muscular Dystrophy (DMD). However, little is known about their involvement in myogenesis in DMD and usefulness as biomarkers in 3*D* platforms for preclinical studies.

Materials and Methods: We performed a pilot study to characterize the electrophysiological asset of myoblasts and myocytes at day 2/4/6/11 using two immortalized mouse cell-lines: the wild-type 2B4 and the dystrophic SF1. We evaluated both inward and outward currents at different time points by whole-cell patch clamp.

Results: 2B4 cells showed an increment of inward currents as the differentiation program progresses. In SF1 myocytes, inward currents increased up to the 6^{th} day of differentiation, like in 2B4 cells. However, day-11 SF1 myocytes showed 50% lower inward currents compared to day-11 2B4 myocytes (5.2 nA vs 2.8 nA at -20 mV). High concentration of tetrodotoxin blocked these inward currents in both day-6 and day-11 2B4/SF1.

Outward currents were clearly detectable in day-11 2B4 cells but very small in day-2/4/6 2B4 cells. Conversely, SF1 outward currents reached the highest value at day-6, being 3-fold higher than 6-day 2B4 cells (733 pA vs 283 pA at +60 mV). However, day-11 SF1 myocytes showed 44% lower outward currents, compared to day-11 2B4 cells (722 pA vs 1283 pA). $BaCl_2$ decreased outward currents in day-6 2B4/SF1 cells and in day-11 2B4 myocytes.

In addition, we assessed resting membrane potentials which became more negative as the days of differentiation increased in both cell-lines. However, day-11 SF1 cells had a more depolarised membrane potential, compared to day-11 2B4 myocytes.

Conclusions: This preliminary data suggests that during myogenesis, intrinsic impairments in ion channel development occurs in dystrophic conditions, likely in relation to the primary defect. The intrinsic alterations support the interest of 2B4 and SF1 cell-lines for preclinical platforms as 3D organoids.

56ASM-0176 | Effects of nitric oxide on the electrical activity of the rat trigeminal nerve

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Background: Migraine is a common pathological condition. The study of the peripheral mechanisms of migraine is relevant for the development of new treatment methods characterized by a directly at the site of the pain signal. However, the molecular mechanisms of pain in the peripheral region remain poorly understood. Nitric oxide (NO) is a member of gasotransmitters family along with CO and H2S. The NO-donor, nitroglycerin, is a trigger of migraine in humans and is used in modeling this disease in animals, which suggests the involvement of the NO signaling cascade in the pathogenesis of migraine. The aim of this work is to analyze the effects of the NO synthesis substrate, L-arginine, and the exogenous NO donor, sodium nitroprusside (SNP), on the electrical activity of the rat trigeminal nerve. We also evaluated the role of guanylate cyclase in the effects of SNP.

Materials and Methods: We used the electrophysiological method for recording AP from trigeminal afferents.

Results: L-arginine dose-dependently increased the electrical activity of the trigeminal nerve (the increase in the frequency of action potentials (AP) was 127.7 ± 38.4% relative to control, $n = 4$, $p < 0.05$) and this effect was blocked by the use of an inhibitor of NO-synthase L-NAME (100 μM, $n = 4$). Exogenous NO donor SNP (200μM) increase the frequency of AP up to 279.3 ± 54.7% ($n = 4$, $p < 0.05$). A selective inhibitor of guanylate cyclase (ODQ, 10 μM) prevented an NO-mediated increase in the frequency of AP in the trigeminal nerve ($p > 0.05$; $n = 4$).

Conclusions: Our results suggests that exogenous and endogenous NO increases the electrical activity of trigeminal nerve indicating on its pro-nociceptive action in the peripheral afferents of the trigeminal nerve. Activation of soluble guanylate cyclase is one of the main mechanisms of NO in trigemino-vascular system.

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56ASM-0181 | In vivo thalamic preparation for electrophysiological recordings in rat pups

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Background: Network maturation during postnatal development is activity-dependent and sensory signals may provide required neuronal activation in the thalamocortical system. The thalamus is a major gateway for sensory outputs to the cortex and its sensory-evoked and intrinsic activities might be crucial for network maturation. The sensory-evoked and spontaneous thalamic activities in newborn animals remain largely unknown. Somatosensory thalamus is located 3-4 mm below the cortical surface in newborn rats which creates technical difficulties in reaching the thalamus with recording electrodes.

Materials and Methods: Experiments were performed on rat pups (6-7 days old) anesthetized with urethane (1-1.3 g/kg, i.p.).

Results: Rat pups were placed in a short tube, put on a warm thermal pad and covered with cottonwool. We placed the pups in a sitting fetal position in the tube so that the head was bent forward relative to the body. The used position allowed the fourth ventricle to be opened in rat pups. To reduce brain pressure and pulsation, we opened the fourth ventricle after surgery. The head was fixed in the stereotaxic frame with metal plates and dental cement. We drilled out a large cranial window above the left hemisphere and gently removed all accessible cortexes and the hippocampus with suction. To reduce pulsation during recordings we filled in the obtained recess with paraffin in mineral oil.

Conclusions: The obtained preparation allowed stable thalamic local field potential, juxtacellular and patch clamp recordings to be performed during 5-6 hours after surgery.

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56ASM-0182 | Anxiety of rats with hyperhomocysteinemia in the model of chronic migraine with aura

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Background: Migraine is a neurological disorder that may associated with endothelial dysfunction caused by an increase in homocysteine levels. Cortical spreading depression (CSD) is a propagated wave of cortical activity that is believed to be the physiological substrate of the migraine with aura and a potential contributor to migraine pain. The aim of our study was the analysis of anxiety of rats with hyperhomocysteinemia in a chronic model of migraine with aura.

Materials and Methods: The study was conducted on male Wistar rats at the age of 5-7 months. Rats were divided to the control group ($n = 6$) and hyperhomocysteinemia group (Hcy) - rats born from females on a methionine diet ($n = 8$). Migraine aura in awaked rats was induced by CSD evoked by the application of KCl on brain dura matter. For the CSD induction 20 μl of KCl solution (1 M) was applied on the dura at the 1st, 3rd, 5th, 7th, and 9th days of the experiment. Anxiety behavior of the animals was analyzed using the light-dark chamber test.

Results: Initially, animals of the Hcy group showed a longer time spent in the dark chamber and a lower latency to enter the dark chamber, which indicates higher anxiety and photophobia compared to the control. At the same time, the number of rearing was lower compared to control. The induction of CSD resulted in the increase of the time spent in the dark chamber and decrease in the latency of the first entry into the dark chamber, and rearing acts in both groups with higher sensitivity of rats from Hcy group.

Conclusions: The obtained results indicate that rats with Hcy demonstrate higher level of anxiety which further aggravates in the experimental model of migraine with aura.

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56ASM-0188 | Age-dependence of neuronal survival after oxygen-glucose deprivation – induced anoxic depolarization in the rat barrel cortex in vitro

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Background: Anoxic spreading depolarization (aSD) is a hallmark of ischemic brain injury. However, the developmental aspects of neuronal death following aSD remain largely unknown.

Materials and Methods: We explored age-dependence of neuronal death as a function of the reperfusion delay after oxygen/glucose deprivation (OGD) - induced aSD in slices of rat barrel cortex during the first postnatal month. The duration of OGD varied according to the delay in reperfusion from aSD peak. We sampled cells using short-term (~ 1 min duration) patch-clamp recordings in control conditions and during reperfusion following OGD starting 20 min after reperfusion. Survived neurons displayed negative resting membrane potential, high membrane resistance and ability to fire action potentials in response to depolarizing current steps. Dead cells lacked resting membrane potential and action potentials, and had low membrane resistance.

Results: Short OGD episodes without aSD were not associated with neuronal death at all ages. If aSD occurred, the number of live neurons progressively decreased with the delay of reperfusion onset after aSD in an age-dependent manner. With an increase in the OGD duration, neuronal death rate attained [median (Q1 – Q3)] 1.3 (0.9 – 1.6) %/min in slices from the postnatal days [P] P1-14 rats and 2.4 (1.9 – 2.6) %/min in P15-32 rats ($p < 0.05$). The level of 90 % death was achieved at post-aSD reperfusion delays of 47 and 32 min at P1-14 and P15-32, respectively.

Conclusions: Thus, (i) the OGD-induced aSD is not a terminal event, (ii) the delay of reperfusion after aSD is critical for neuronal survival and (iii) the rate of neuronal death during post-aSD period increases with age.

This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

56ASM-0217 | Non-immunogenic polymeric nanoparticles as an efficient brain-blood barrier targeting agent

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Background: There are still no available therapies to reactivate brain's function after occurrence of neurodegenerative diseases. There is an urgent need to develop an effective drug carrier capable of crossing the blood-brain barrier (BBB) and promoting the therapeutic brain accumulation of CNS-targeting drugs. The transient opening of the BBB using photothermal energy-generated nanoparticles (NPs) upon near-infrared (NIR) light exposure has gained tremendous interest in the context of brain targeting. It remains elusive if the photothermal energy produced by NPs activates a pro-inflammatory response which might affect the BBB function and integrity. Here we report a polymeric formulation suitable for an efficient BBB crossing without triggering immunotoxicity.
Materials and Methods: We have studied the combined effect of transferrin (Tf) peptide and photothermal energy produced by polydopamine (PDA) NPs on crossing ability through bEnd.3 cells monolayer model.
Results: Our results show the conjugation of 180 Tf peptides per PDA NP (Tf_{180}-PDANPs). Upon NIR light exposure (785nm, 2W/cm^2, 5min), PDA NPs increase the temperature 5ºC. We demonstrate that the photothermal energy generated by PDANPs do not induce M1 macrophage transition and subsequent secretion of cytokines. Importantly, conditioned media obtained from macrophages (exposed to NPs and/or NIR light) do not affect the integrity of BBB model. bEnd.3 cells internalized higher percentage of Tf_{180}-PDA NPs upon NIR light exposure compared to Tf_{180}-PDA NPs/PDA NPs without light exposure. Importantly, both NP and light exposure do not affect the bEnd.3 cells monolayer integrity. The results show that Tf_{180}-PDA NPs cross the monolayer more efficiently than the PDA NPs upon NIR light exposure. Additionally, we show that PDA NPs elicit no acute activation of macrophages after NIR light exposure, causing no immunotoxicity in the BBB model.
Conclusions: Overall, PDA NPs could be used as effective light-responsive carriers to deliver drugs for treating multiple CNS disorders. This work was funded by FCT investigator grant (IF/00539/2015).

SYMPOSIUM 7: REGENERATIVE – GENOMIC MEDICINE

56ASM-0287 S7 IS | RESEarch for healThy AGEING – RESETageing – a twinning project

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As the human expectancy within the European Union continues to increase, the impact of chronic diseases and their dependencies also increase leading to an enormous burden to individuals and to societies. The healthy life expectancy, which is the number of years spent free of disabilities, grows much slower than life expectancy, and this growing has been inequal between EU countries, being Portugal one of the countries where the healthy life expectancy growing has been less significant.
To reduce these gaps in healthy life expectancy, interventions that increase healthy ageing are needed and probably are the most promising potential to relieve individual suffering as well as the enormous strain on public finances. RESETageing project aims at enhancing the scientific and innovation competences of University of Coimbra (UC), Portugal, a low-performing partner, in the area of cardiovascular ageing, with three high performing partners, University of Newcastle upon Tyne (UNEW), United Kingdom (ageing biology), the University of Maastricht (UM), Netherlands (cardiovascular biology) and the Leibniz Institute on Aging– Fritz Lipmann Institute (LIA), Germany (omics).
The objectives of RESETageing project are to stimulate the excellency in research, training and value creation in the area of ageing.
Keywords + Funding: Ageing; Human induced pluripotent stem cells; Disease modelling; HGPS;
European project RESETageing (ref. 952266)

56ASM-0023 | Spinal cord disorders in mouse model of amyotrophic lateral sclerosis during development of diseases

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Background: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease of motor neurons. Studying the structure of spinal cord at various stages, while the disease progresses, is urgent, in order to understand which cells should be targeted to gain positive impact of therapy.
Materials and Methods: Using transmission electron microscopy, we examined the cross sections of ventral horns of spinal cord of ALS mice at the various stages of the disease, as well as spinal cord of wild-type mice. Using immunofluorescent confocal microscopy, we performed morphometric and quantitative analysis of glial cell populations.
Results: At the stage of preclinical manifestations, the integrity of tissues decreases. At the terminal stage of the development of the disease, the gray matter is disintegrated and looks homogeneous. At the stage of clinical manifestations, there is a sharp increase in the number of reactive astrocytes, which continues until the terminal stage of the disease. Activation of microglia and a significant increase in the number of activated microglial cells by 3.4 times occurs only at the terminal stage of disease. The number of oligodendrocytes decreases gradually and single oligodendrocytes are visualized at the terminal stage. By the end stage, the number of NG2+ cells decreases, but the number of astrocytes expressing NG2 proteoglycan increases significantly.
Conclusions: Thus, reactive astrogliosis occurs already at the preclinical stage of ALS, which is characterized by a change in the morphology of astroglial cells and the acquisition of a neurotoxic phenotype by them. As the disease progresses into the next stage, cytokines secreted by reactive astrocytes activate NG2 cells, which respond by increased proliferation. However, denervated oligodendrocytes die, and at the terminal stage, activated microglia proliferate and phagocytize the degenerated cells. As a result, a glial scar is formed: astrocytes-NG2 cells-microglia.

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56ASM-0032 | Effect of intranasal administration of mesenchymal stem cells on the nitric oxide content in the olfactory bulbs after modeling of ischemic stroke

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Background: It is known that hypoxia is accompanied by disturbances in the oxygen supply to the brain, which can cause cerebral ischemia, which may culminate in ischemic stroke. No doubt that the role of nitric oxide (NO) system in the pathogenesis of a number of diseases associated with vascular disorders is defining. In the past few decades, evidence has emerged that physiological renewal and tissue regeneration throughout the life of an animal and a human occurs through stem cells, the most important of them are mesenchymal stem cells (MSCs). The aim of this work was to study the intensity of NO production in the olfactory bulbs of the brain after modeling ischemic stroke, and the effect of intranasal administration of MSCs in the acute period after modeling of ischemia.
Materials and Methods: The modeling of the ischemic stroke was carried out by obstruction of the common carotid arteries. The detection and quantification of NO in olfactory bulbs was made using electronic paramagnetic resonance (EPR) spectroscopy.
Results: The signal of EPR spectrum of olfactory bulb showed the characteristic triplet signal with a g-factor equal to 2.038. It was found a significant reduction of NO content in the olfactory bulb of the brain of rats on the 1st and 2nd days after modeling of ischemia caused by ligation of the carotid arteries. The level of NO production was also reduced on the 1st and 2nd days after ischemia in rats after modeling of the ischemia with immediate intranasal administration of MSCs as compared to with the group of intact animals. It was not found the significant difference of the NO content in rats after modeling of the ischemia with immediate intranasal administration of MSCs relative to ischemic rats.
Conclusions: The experiments showed that intranasal administration of MSCs did not affect the intensity of NO

production on the 1st and 2nd days after the modeling of brain ischemia.

Key words: mesenchymal stem cells, nitric oxide, approximate motor activity, ischemic brain stroke, EPR spectroscopy

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56ASM-0064 | Uptake of induced microvesicles by human blood mononuclear cells

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Background: Protective function of peripheral blood mononuclear cells (PBMCs) is based on their ability to destroy damaged or foreign cells, perform antigen presentation, secrete various antibodies and cytokines. Blood cells are the first to meet therapeutics after an intravenous infusion. In this work we produced biocompatible microvesicles using cytochalasin B and investigated the ability of various populations of human PBMCs (monocytes, NK-cells, B-cells, T-cytotoxic, T-helper lymphocytes) to uptake cytochalasin B induced microvesicles derived from mesenchymal stem cells (CIMVs-MSCs).

Materials and Methods: PBMCs were isolated from anticoagulated blood using Ficoll (PanEco, Russia) gradient centrifugation. CIMVs were obtained from human adipose tissue mesenchymal stem cells by treatment with 10 μg/ml cytochalasin B (Sigma-Aldrich, USA) for 30 min followed by centrifugation: 100 g for 10 min, 300g for 20 min and 2000g for 25 min. CIMVs-MSCs were stained with membrane dye DiD 5μM (Life Technologies, USA) 15 min (37 °C, 5% CO2) and washed twice with complete medium. PBMCs were treated with 10 μg CIMVs for 24h and analyzed using flow cytometer BD FACS Aria III (BD Bioscience, USA).

Results: We revealed that 99.5 ± 0.26% of monocytes (CD14+ cells), 29.6 ± 3.2% NK-cells (CD3-CD56+); 69.43 ± 9.52% B-cells (CD3-CD20+); 35.6 ± 3.83% T-cytotoxic lymphocytes (CD3+CD8+) and 14.5 ± 4.42% T-helper lymphocytes (CD3+CD4+) - were positive for DiD. The obtained results were confirmed by confocal microscopy. We observed membrane component of CIMVs which was stained with DiD on cell surface and inside of PBMCs.

Conclusions: Our data demonstrate that monocytes and B-cells are preferentially uptake CIMVs-MSCs, while NK-cells, T-cytotoxic lymphocytes and T-helpers show less ability to capture induced microvesicles.

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56ASM-0065 | Induced microvesicles inhibit proliferation of human peripheral blood mononuclear cells

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Background: Extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) retain the immunosuppressive potential of parental cells that makes them a promising therapeutic instrument for the treatment of various immune-mediated diseases. However, the yield of EVs is limited and methods of increased production/isolation of EVs are being actively developed. In our work, we use cytochalasin B-induced microvesicles (CIMVs) from MSCs, which show an increased yield. The aim of our work was to investigate the immunomodulatory activity of CIMVs derived from MSCs in a model of phytohaemagglutinin (PHA)-induced activation of human peripheral blood mononuclear cells (PBMCs) *in vitro*.

Materials and Methods: PBMCs proliferation was analyzed using a fluorescent dye CFDA SE (10 μM). PBMCs were preincubated with CIMVs for 24 hours and then treated with 10 μg/ml PHA for 72 hours. Percent of proliferated PBMCs was analyzed using flow cytometry.

Results: Our results show that treatment of PMBCs with CIMVs-MSCs did not significantly affect the percent of proliferating cells (9 ± 1.2% - non treated PBMC versus 6,5 ± 2% - CIMVs treated PBMCs). As expected, PHA induced PBMCs proliferation (43.1 ± 5.9% proliferating cells versus 9 ± 1.2% - in control; $p = 0{,}00034$). While pretreatment of PBMCs with CIMVs significantly decreased the percentage of activated and proliferating PBMCs (15.35 ± 0.6% versus 43.1 ± 5.9% in PHA treated cells; $p = 0{,}0041$). We found that PHA stimulated the proliferation of CD4+, CD19+, CD8+ leukocytes by 91.2, 18.6 and 39.3 times more compared to the control. Pretreatment of PBMCs with CIMVs-MSCs led to suppression of proliferation of CD4+, CD19+, CD8+ cells by 2.9, 2.1 and 2.9 times, respectively.

Conclusions: We have demonstrated that pretreatment of PBMCs with MSCs derived CIMVs significantly inhibits PHA-activated proliferation of lymphocytes. This work was part of Kazan Federal University Strategic Academic

Leadership Program (PRIORITY-2030) and funded by RSF according to the research project №21-75-10035.

56ASM-0067 | Influence of storage conditions on integrity of cytochalasin b induced microvesicles

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Background: Microvesicles (MVs) are spherical micro- and nanostructures surrounded by a membrane that participate in intercellular communication. Today, MVs derived from mesenchymal stem cells (MSCs) are promising tools for the treatment of various diseases. Treatment of cells with cytochalasin B makes it possible to obtain MVs in a larger amount suitable for the industrial scale. However, stability of MVs in a storage solution has not been studied previously. In this regard, the aim of our work was to evaluate stability of cytochalasin B induced MVs (CIMVs) under different storage conditions.

Materials and Methods: CIMVs were prepared as described previously. Isolated MVs were resuspended in saline, then divided into separate tubes and placed in various storage conditions: 1) at +4°C for up to 112 days; 2) at -20°C for up to 112 days; 3) at 25°C for up to 28 days; 4) freeze dried and stored at -20°C for 112 days. We estimated protein concentration in the CIMVs storage solution using the pierce TM BCA Protein Assay Kit (Thermo Scientific, USA).

Results: The initial protein concentration in the storage solution at day 0 was 28.65 ± 12.1 μg/mL. After 1 day of storage, the protein concentration in the solution increased to 99.6 ± 13.6 μg/mL (storage in saline t +4°C), 128.5 ± 23.2 μg/ml (storage in saline at -20°C), 138.1 ± 18.7 μg/ml (storage in saline at +25°C), 199.2 ± 18.4 μg/ml (freeze drying/rehydration). After 112 days of storage, the protein concentration in the solution increased to 119.7 ± 45.2 μg/ml (saline at +4°C), 87 ± 14.7 μg/ml (saline at -20°C), 176.3 ± 16.8 μg/ml (freeze drying/rehydration).

Conclusions: We have shown for the first time that the most suitable storage condition for CIMVs is in saline at -20°C.

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56ASM-0073 | The study of tumor spheroids formation after cytochalasin B induced membrane vesicles addition

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Background: Tumor microenvironment plays an important role in cancer progression. Understanding the mechanisms of interaction between tumor cells and its microenvironment can help to treat oncological diseases. Cells can interact with each other through extracellular vesicles, which include membrane vesicles or microvesicles (MVs). The aim of this study is to investigate the effect of different MVs on tumor spheroids in vitro.

Materials and Methods: In this study, breast cancer cells (MCF-7) were used to create tumor spheroids. Glioblastoma cell line (SNB-19) and adipose derived mesenchymal stem cells (MSCs) were used to isolate MVs. Vesicles from SNB-19 (SNB-19 IMVs) and MSCs (MSC CIMVs) were isolated using 10 μg/ml of cytochalasin B and a series of sequential centrifugations. The addition of SNB-19 CIMVs and MSC CIMVs to spheroids was carried out at concentrations of 5, 10 and 20 μg. The effect of SNB-19 CIMVs and MSC CIMVs was analyzed using confocal microscopy, flow cytometry, multiplex analysis, and real-time PCR.

Results: SNB-19 CIMVs and MSC CIMVs were found to be fused with MCF7 cells after 24 hours of cultivation. The addition of SNB-19 CIMVs and MSC CIMVs increased the number of tumor spheroids. However, 20 μg of MSC CIMVs decreased cell viability on the fourth day of spheroid culture. SOX2 and OCT4 mRNA levels were increased after adding SNB-19 CIMVs and MSC CIMVs.

Conclusions: Thus, it was shown the possible effect of SNB-19 CIMVs and MSC CIMVs on tumor spheroids. Further research of this mechanism effects is necessary.

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56ASM-0074 | Cytochalasin B-induced membrane vesicles from mesenchymal stem cells overexpressing TRAIL activate key components of the apoptotic pathway in breast cancer mouse model

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Background: TNF-related apoptosis-inducing ligand (TRAIL) selectively induces apoptosis in tumor cells by activating TRAIL-mediated apoptosis inducing pathway. Transport of TRAIL in extracellular vesicles is believed to be highly efficient method for TRAIL delivery to the tumor microenvironment and apoptosis induction.
Materials and Methods: In this work induced membrane vesicles were isolated from native Wharton's jelly mesenchymal stem cells (WJ-MSCs) (native CIMVs) or WJ-MSCs over-expressing TRAIL using Cytochalasin B treatment (CIMVs-TRAIL). To study the antitumor effect of CIMVs-TRAIL *in vivo*, a breast cancer mouse model was produced. The animals were injected in the tumor area with 50 μg of native CIMVs or CIMVs-TRAIL in 20 μl of PBS. Injections were carried out for 12 days with an interval of two days. Then the mice were euthanised, total RNA was isolated from the tumour tissue for subsequent analysis of the expression of apoptotic genes *CAS8; BCL2* and *BAX* using qPCR.
Results: A 1.8-fold increase in the *CAS8* gene mRNA level was observed in the tumours of mice received CIMVs-TRAIL compared to control animals. The expression of the anti-apoptotic *BCL2* gene in the CIMVs-TRAIL group remained unchanged, while the mRNA level of the pro-apoptotic *BAX* gene was increased by 1.4 times, which indicates activation of the apoptotic cascade and induction of tumor cell death.
Conclusions: Thus, it has been shown that CIMVs-TRAIL are able to activate apoptotic signaling pathway and induce tumor cell death in the breast cancer mouse model. Thus, the use of CIMVs-TRAIL can be considered as a promising strategy for cell-free therapy of breast cancer.
This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and was funded by the Russian Science Foundation grant 18-74-10044.

56ASM-0088 | The study of colonosphere formation after the addition of cytochalasin B induced membrane vesicles

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Background: Extracellular vesicles are membrane structures that have a relevant role in intercellular communication because they have the ability to transport lipids, transcription factors, mRNA, and proteins. There is evidence of a special role of vesicles in cancer progression. Therefore, the study of the vesicle effect on tumor spheroids is important. The aim of this work is to study the effect of cytochalasin B-induced membrane vesicles (CIMVs) of colorectal cancer spheroids *in vitro*.
Materials and Methods: In this study, colorectal cancer cell line (HCT-15) was used to create tumor spheroids. Glioblastoma cell line (SNB-19) and adipose derived mesenchymal stem cells (MSCs) were used to isolate MVs. Vesicles from SNB-19 (SNB-19 CIMVs) and MSCs (MSC CIMVs) were isolated using 10 μg/ml of cytochalasin B and a series of sequential centrifugations. The addition of SNB-19 CIMVs and MSC CIMVs to spheroids was carried out at concentrations of 1, 2 and 5 μg. The effect of SNB-19 CIMVs and MSC CIMVs was analyzed using flow cytometry, multiplex analysis, and real-time PCR.
Results: After addition of MSC CIMVs, there was no significant change in cell viability in the spheroid. However, addition of SNB-19 CIMVs to colonosphere, there was a dose-dependent increase cell viability. OCT4 and Nanog mRNA levels were increased after addition of SNB-19 CIMVs but decreased after MSC CIMVs. Cytokine analysis showed significant differences in 40 major cytokines in colonospheres with SNB-19 CIMVs and MSC CIMVs.
Conclusions: Thus, it was shown the possible effect of SNB-19 CIMVs and MSC CIMVs on tumor spheroids.
This study was part of the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by the Russian Science Foundation grant 21-74-10021.

56ASM-0091 | Analysis of immune cell activation after interaction with membrane vesicles from genetically modified tumor cells

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Background: Cytochalasin B induced membrane vesicles (CIMVs) are membrane-bound structures of various sizes, that contain proteins, lipids, nuclear and mitochondrial components. CIMVs are able to fuse with recipient cells via endocytosis. Therefore, CIMVs from tumor cells can be used to present antigens to cells of the immune system.
Materials and Methods: Genetic modification of human melanoma M-14 cells was carried out with recombinant lentiviruses LV-IL-GM-CSF to obtain stable cell lines with overexpression of GM-CSF. Peripheral blood monocytes (PBMCs) were isolated by Ficoll gradient centrifugation (1,077 g/cm^3). Differentiation of DCs from PBMCs was reached by cultivation of PBMCs with a cocktail of cytokines for 7 days. Co-cultivation of DCs and CIMVs was carried out for 24 hours, and then PBMCs were added. Afterward PBMCs were stained with the antibodies containing a fluorescent label. The results were analyzed by flow cytometry.
Results: We analyzed the interaction of activated mature DCs with PBMCs. Co-cultivation of dendritic cells loaded with CIMVs from M-14-GM-CSF with PBMC resulted in an increase in CD38$^+$ T-killers by 32% relative to PBMCs that were cultured with native mature DCs (control cells), as well as an increase in T-helper 2 cells by 17% relative to control cells. It was shown a statistically significant *p*-value difference 0.0017 and 0.04, respectively.
Conclusions: Thus, due to the ability of CIMVs to present tumor antigens to DCs and activate the antitumor immune response, CIMVs of tumor cells are a promising object for the development of therapeutic antitumor vaccines. However, further studies are needed in this area to study possible ways of modulating the immune response.
This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by the Russian Science Foundation grant 22-24-20018.

56ASM-0129 | Novel Small molecules with capability to reactivate mutant p53

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Background: The p53 protein is a tetrameric transcription factor that preserves the integrity of the genome by activating downstream genes whose protein products are involved in cell cycle arrest, apoptosis and DNA repair. The *TP*53 gene is one of the most frequently mutated genes in human cancers. The presence of a mutation leads to destabilization of the tertiary structure of the p53 protein and, as a consequence, disruption of its transcriptional activity. Also, the protein mutation causes uncontrolled cell proliferation, inhibits apoptosis, confers resistance to specific anti-cancer drugs, promotes invasion and metastasis. Reactivation of mutant p53 with small molecule compounds holds good promise as a therapeutic strategy for treating a large number of human tumors. The aim of this research was to study the biological properties of derivatives of previously reported MB725 considered as selective small-molecule stabilizers of the p53(Y220C) mutant.
Materials and Methods: Cell lines containing wild-type p53 or mutant p53(Y220C) were exposed to derivatives of MB725 for 24 and 48 h. The rate of apoptosis was measured by flow cytometry assay using Annexin V/ Propidium Iodide. Immunocytochemistry analysis and western blotting were used for evaluation of the expression levels of p53-dependent proteins.
Results: Our data revealed that MB725 derivatives induced apoptosis in HUH7 p53(Y220C) cell line. In addition, compounds induced an increase in p53-dependent protein levels in cell lines with mutant p53.
Conclusions: MB725 derivatives were confirmed as highly potent reactivators of p53 functions in mutant p53(Y220C) cells.
The study was funded by RSF grant 19-74-10022 and strategically supported by Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0130 | Activation of Mutant Tumor Suppressor p53 Under the Influence of Small Molecule Modulators

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Background: The tumor suppressor protein p53 is a key link in numerous important cellular processes, including cell cycle regulation, cell differentiation and metabolism, DNA repair and apoptosis. Studies show that inactivation of p53 in almost all cases is associated with tumor progression, which makes it the primary target for cancer therapy. Oncogenic mutation $p53^{Y220C}$ creates an expanded surface pocket in the DNA-binding domain, which destabilizes p53. Reactivation of mutant p53 using small molecule compounds derived from previously reported MB725 is a promising strategy for the development of new antitumor therapeutics.

Materials and Methods: In this work the following cell lines MCF7 (p53wt), MCF7 ($p53^{-/-}$), MCF7 ($p53^{Y220C}$) and HUH7 ($p53^{Y220C}$) were treated with compounds at the concentration range of 0–200 uM. Cell viability was evaluated using colorimetric MTS test. The expression of p53 target genes such as *PUMA*, *p21; MDM2* upon treatment with the compounds was also quantified using real-time quantitative PCR.

Results: Semi-inhibitory concentrations were determined on the studied cell lines. IC50 values for HUH7 $p53^{Y220C}$ and MCF7 $p53^{Y220C}$ cell lines were determined in the range of 32–55 uM, while in cells with wild-type p53 status and with *TP53* knockout IC50 values were significantly higher. Evaluation of the compounds effect on expression of p53 target genes in cells lines HUH7 and MCF7 $p53^{Y220C}$ was also analyzed. We established that the expression of *PUMA* increased in HUH7 and MCF7 $p53^{Y220C}$ cells treated with the compounds.

Conclusions: The results demonstrated that the compounds exert specific action towards the cells carrying $p53^{Y220C}$ mutation. We also found that the compounds selectively reduce cell viability in $p53^{Y220C}$ cell lines and upregulate transcription of p53 target genes associated with apoptosis in a $p53^{Y220C}$-dependent manner.

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56ASM-0131 | Cytological pattern, HPV-status and expression of p16ink4a protein in cervical cancer diagnostics

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Background: Most cases of cervical cancer are associated with HPV infection. However, not every case of HPV infection results in cervical cancer. This is due that HPV can be found inside the cell in two forms: latent when the virus is inactive, and active, when the virus is embedded in the cell genome and triggers a cascade of reactions. The signal of embedding HPV in the cell genome is expression of regulatory proteins, the main of which is p16in4a, cyclin dependent kinase.

This study was conducted to assess the correlation of the cytological pattern, HPV status and expression of the p16INK4A in the diagnosis of cervical cancer.

Materials and Methods: A total of 852 women were enrolled in the study. A pap smear test was performed by using liquid cytology. Two smears were performed for one patient: one for cytological screening and one for immunocytochemical analysis expression p16ink4a. HPV status results were known in 796 cases.

Results: The Pap smear test was negative for malignancy in 83% cases. Atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) were detected in 9,2%, 6,4%, and 0,8%, respectively. Statistical analysis of the results has shown that the immunocytochemical analysis allows to reliably distinguish from the group with ASCUS and LSIL ($p < 0{,}005$), patients with negative prognosis. HPV PCR data showed that the percentage of HPV infested in the ASCUS and LSIL increased unreliably ($p > 0{,}5$).

Conclusion: Cytological examination allows to diagnose visible alteration epithelial conditions, but they do not reflect the impact of HIV infection on disease development before any visible signs of neoplasia. Immunocytochemical analysis expression of p16ink4a have the highest accuracy in detection of molecular changes in cells and predict their further course.

The work was supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0135 | Molecular design of novel NKG2D chimeric antigen receptor and its ligands

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Background: Despite the success of chimeric antigen receptor (CAR)-T therapy in hematological malignancies, for solid tumors results remain limited. For CAR-T therapy searching for universal but highly specific tumor antigen is the key to effectiveness and safety. A promising candidate is a pair of NKG2D receptor and its ligands (MICA, MICB, ULBP2-6) as NKG2D is expressed on NK-cells and some populations of CD8+ T-cells, providing recognition and elimination of cancer cells, and NKG2DLs gets dramatically increased due to cancer transformation or treatment. In this study, we performed the design of NKG2D-CAR and NKG2DLs, followed by the demonstration of high expression of NKG2DLs using in vitro cell-based models.

Materials and Methods: Lentiviral plasmid vector pLVT was used for CAR. The following constructs were cloned into this vector: protein-coding sequence of NKG2D fused to CD3z-signaling domain, protein-coding sequence of Dap10 adapter protein, and sequence of suicide cassette based on truncated EGFR. For NKG2DLs constructs were based on third-generation lentiviral plasmid vector pUltra-hot plasmid #24130 (Addgene) and mCherry reporter protein was replaced with selective blasticidin resistance gene by molecular cloning. The validity of the resulting vectors was verified by restriction. Obtained vectors were used for lentiviral transduction of target cells (primary T-cells for NKG2D-CAR and HeLa for NKG2DLs) with confirmation of expression by flow cytometry (NKG2D-CAR) and qPCR (HeLa).

Results: We have successfully obtained a plasmid vector encoding NKG2D-CAR and generated NKG2D-CAR-T-cells (19,58% transduction efficacy). We also designed a series of NKG2DLs plasmids and generated a modified HeLa cell line overexpressing NKG2DLs: MICA (200x higher than control), ULBP2 (200x higher than control), ULBP6 (77000x higher than control).

Conclusions: Further experiments will evaluate the effectiveness of these NKG2D-CAR-T-cells against tumor cells overexpressing NKG2D ligands.

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56ASM-0137 | Functional assessment of cytotoxic activity of NKG2D CAR-T cells

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Background: NKG2D is an activating transmembrane receptor presented on the surface of NK or T cells. NKG2D CAR recognizes not only tumor cells, but also immunosuppressive cells such as myeloid suppressor cells and regulatory T cells, as well as endothelial cells in the tumor microenvironment. NKG2D CAR-T cells eradicate tumor cells and produce numerous cytokines. The aim of this study was to study the cytotoxic activity of CAR-T cells in real-time dynamics using RTCA methodology, as well as to assess the release of interferon-gamma during co-cultivation of tumor cells and CAR-T cells.

Materials and Methods: The cytotoxic activity of CAR-T cells was assessed in real time during co-cultivation with the HeLa line using the RTCA xCelligence (ACEA Biosciences). The evaluation of cytotoxic effect of CAR-T cells was carried out within 72 hours of co-cultivation at a ratio of E:T = 1:1. Effector CAR-T cells were seeded after 24 hours of target tumor cell culture. The functional cytotoxic activity of CAR-T cells was also assessed using ELISA (Vector-Best) to identify the concentration of interferon-gamma in co-cultivation supernatant.

Results: The results revealed that NKG2D CAR-T cells possess a significant cytotoxic effect against HeLa cells overexpressing NKG2D ligands. According to ELISA, co-cultivation of NKG2D CAR-T effectors with HeLa cells leads to increased levels of interferon-gamma. However, these levels are lower than for anti-CD19 CAR-T cells co-cultivated with HeLaCD19+.

Conclusions: ELISA results are in good agreement with RTCA data and indicate a significant NKG2D CAR-T-mediated antitumor cytotoxic effect.

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56ASM-0138 | Effect of p53 activator nutlin-3a on cytokine release in peripheral blood mononuclear cells of healthy donor and patient with multiple sclerosis

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Background: The role of p53 in autoimmune diseases was demonstrated in many models. Multiple sclerosis (MS) is a chronic autoimmune disease targeting myelin. At the moment, there are 44 cases of MS per 100,000 population in the world and the number of patients is constantly increasing. However, the role of p53 in MS regulation is still unclear. In this study, we explore the influence of p53 activator Nutlin-3a on the secretion of cytokines by immune cells of healthy donor and MS patient.

Materials and Methods: In this study we used peripheral blood mononuclear cells (PBMCs) separated using Ficoll gradient from whole blood of healthy donor and MS patient. PBMCs from each donor were divided into 3 groups: untreated, treated with 10 μM and 40 μM Nutlin-3a. PBMCs were incubated with Nutlin-3a for 24 h. Cell supernatants were collected and tested by multiplex assay using Bio-Plex 200 system (Bio-Rad, USA). To evaluate the effect of Nutlin-3a on p53 activation and expression of p53-dependent genes we isolated mRNA from PBMCs of each group and estimated the expression levels of p21; Bax, PUMA by RT-PCR.

Results: RT-PCR analysis demonstrated increased expression levels of p53-dependent genes (p21; Bax, PUMA). According to the multiplex cytokine analysis, Nutlin-3a in healthy donor increased IP-10 level, decreased levels of IL-10; G-CFS, MCP-1; TNF-α, VEGF and did not affect IL-1b, IL-1ra, IL-15; INF-γ, RANTES levels. In MS patient, Nutlin-3a increased levels of IL-1b and TNF-α, decreased IL-1ra, IL-10; G-CSF, VEGF and did not affect IL-15; IFN-γ, IP-10; MCP-1; RANTES.

Conclusions: Our results demonstrate that p53 activator Nutlin-3a affects cytokine secretion in PBMCs of healthy donor and MS patient. This allows the assumption that p53 protein is involved in the regulation of immune processes.

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56ASM-0150 | Cross-correction of β-hexosaminidase a deficiency using genetically modified human mesenchymal stem cells in a cell culture of tay-sachs disease patient

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Background: GM2-gangliosidoses are autosomal recessive diseases caused by impaired enzymatic activity of β-hexosaminidase A (HexA); an essential enzyme for metabolism of GM2-gangliosides and other molecules containing terminal N-acetyl hexosamines. HexA deficiency causes accumulation of GM2-gangliosides mainly in the nervous system cells, leading to severe progressive neurodegeneration. Gene-cell therapy is considered a promising treatment for GM2-gangliosidoses. This study aimed to evaluate the possibility of restoring HexA deficiency in cells of Tay-Sachs disease (TSD) patient using genetically-modified mesenchymal stem cells (MSCs).

Materials and Methods: MSCs were isolated from human adipose tissue, and genetically modified with recombinant adeno-associated viruses encoding codon-optimized cDNA of α- (*HEXA*) and β-subunits (*HEXB*) genes of HexA (MSCs-HEXA-HEXB). MSCs-HEXA-HEXB were cultured with TSD patient's MSCs (mutMSCs) in a transwell system. After 7 days, the effectiveness of HexA deficiency cross-correction was studied. HEXA concentration in cell lysates was determined by ELISA. Copy number of *HEXA* and *HEXB* genes was determined using qPCR. Detection of HEXA and HEXB proteins was carried out by ICC.

Results: After HexA delivery by cross-correction in the transwell culture system, mutMSCs contained 72903.12 ± 14026.65 and 80899.7 ± 20847.92 copies of codon-optimized HEXA and HEXB genes, respectively, per 1 μg of total RNA. HEXA concentration in mutMSCs after cross-correction of HexA deficiency (0.71191 ± 0.069171 ng/μl) increased by 2.25-fold compared to native mutMSCs (0.302794 ± 0.0273096 ng/μl). Both α- and β-subunits of HexA were detected in mutMSCs using ICC after enzyme deficiency cross-correction.

Conclusions: Therefore, the effectiveness of HexA deficiency cross-correction was shown in TSD patient's mutMSCs upon interaction with MSCs-HEXA-HEXB. Such correction could be mediated by vesicular transport and HexA delivery via extracellular vesicles MSCs-HEXA-HEXB.

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56ASM-0170 | Extracellular Vesicles Secreted by Mononuclear Cells from Mmbilical Cord Blood Induce Neuroprotection and Angiogenesis and Attenuate Microglia Reactivity in a Mouse Model of Brain Ischemia

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Background: Extracellular vesicles (EVs) have emerged as a promising therapy for ischemic stroke. Although recent evidence points that EVs have the ability to cross the blood brain barrier [1], the majority of the injected EVs accumulate in extra-cranial organs[2], suggesting that most of the effects that have been observed in ischemic stroke models might be systemic and not local effects. Thus it is still unclear to which extent EVs have a direct effect in the brain parenchyma after ischemia
Materials and Methods: Herein, we investigated *in vitro* and *in vivo* the therapeutic potential of EVs, focusing on their local effect in the brain parenchyma. We started by evaluating the pro-survival effect of EVs secreted by Wharton-Jelly mesenchymal stem cells (MSCs) and by human umbilical cord blood mononuclear cells (MNCs) in endothelial cells and neurons as well as their effect in microglia reactivity and the uptake mechanism in microglia. We further investigated these effects *in vivo* after stereotaxic injection in a mouse model of brain ischemia induced by medial cerebral artery occlusion.
Results: Both MNC-EVs and MSC-EVs were able to increase the survival of endothelial cells. On the other hand, only MNC-EVs could increase the survival of cortical neurons and reduce the reactivity of microglia after exposure to oxygen and glucose deprivation conditions. Uptake experiments revealed that microglia internalize preferentially MNC-EVs and that the uptake is partially mediated by MHC-II present on MNC-EVs surface. Likewise, MNC-EVs were preferentially taken up by microglia *in vivo* at 6h after stereotaxic injection and decreased microglia reactivity 3 days after treatment. MNC-EVs were also able to decrease cell death, as evaluated by TUNEL assay, and increase the number of proliferative endothelial cells.
Conclusions: Overall, MNC-EVs have a direct effect in different brain cells and show higher neuroprotective and regenerative properties than MSC-EVs in the context of brain ischemia.

56ASM-0197 | bFGF regulates adipose-derived stem cells from different fat depots in lean and obese subjects to differentiate into endothelial cells by a specific microRNA signature

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Background: Adipose tissue (AT) is a heterogeneous and multi-depot tissue containing adipose-derived stem cells (ASCs). These undifferentiated progenitor-cell population can differentiate into multiple cell lineages, including endothelial cells (ECs). MicroRNAs (miRNAs) are key regulators of the endothelial differentiation process. However, key events that contribute to the angiogenic properties of ASCs remain to be fully elucidated. Our hypothesis is that bFGF regulates an angiogenic miRNA profile involved in the differentiation of ASCs into ECs by a defined genetic signature in AT fat depots.
Materials and Methods: Human ASCs from subcutaneous AT (SAT-ASCs) and visceral AT (VAT-ASCs) were obtained by surgical procedures from lean and obese invidious. Importantly, SAT-ASCs and VAT-ASCs were obtained from the same obese patient. ASCs were differentiated into ECs with 10ng/ml bFGF during 9 days. miRNA array was used to analyse the expression of 4603 mature and immature miRNA sequences in four biological replicates of bFGF-differentiated ASCs. By *in-silico* analysis differentially expressed miRNAs with a threshold of p-value < 0.05, $\log_2(FC) \pm 6$, and angiogenic properties were validated by qRT-PCR. The expression of EC markers was confirmed for bFGF- as well as mimic/inhibitor-differentiated ASCs.
Results: Significant up- and down-regulated miRNAs were identified from each AT fat depot. The *in-silico* analysis indicated that the miRNA profile of SAT-ASCs from lean subjects mostly contained up-regulated miRNAs, while both SAT-ASCs and VAT-ASCs from the same obese subject mainly included down-regulated miRNAs. This analysis also showed that SAT-ASCs, independent of BMI status, contained higher amounts of differentially expressed miRNAs in comparison to VAT-ASCs. We have identified 4 miRNAs involved in the angiogenic properties of ASCs.

Conclusions: In summary, bFGF induces ASC to EC differentiation showing that ASC endotheliogenesis involves a distinctive miRNA signature relying on the AT depot.

56ASM-0206 | The FAPx4-1BB bispecific DARPin® Molecule MP0310: enhances daratumumab-mediated anti-myeloma activity

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Background: Daratumumab is an anti-CD38 mAb with potent anti-multiple myeloma (MM) activity through antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells. Despite promising results, MM patients treated with daratumumab invariably relapse. MP0310 is a bispecific a DARPin® molecule that simultaneously binds fibroblast activation protein (FAP) and 4-1BB (CD137), resulting in CD137 clustering and signaling. CD137 is a co-stimulatory receptor expressed on activated NK cells. Fibroblasts (FBs) from patients with MM overexpress FAP and contribute to drug resistance by creating a supportive bone marrow (BM) niche. Since in the BM microenvironment MM cells adhere to FBs, MP0310 may lead to further recruitment of activated NK cells towards FBs and fostering daratumumab-mediated NK cell cytotoxicity against MM cells. Here, we evaluate the effect of MP0310, on the daratumumab-mediated ADCC.
Materials and Methods: CD137; CD107a and perforin expression on NK cells was evaluated by FACS analysis of BM mononuclear cells from MM patients at different clinical stages. The effect of MP0310 on the daratumumab-mediated cytotoxic activity was performed by evaluating ADCC against the human $CD38^+$ RPMI8226 and $CD38^-$ U266 MM cell lines using the calcein-AM release assay. ADCC was evaluated using daratumumab pre-treated and untreated BM lymphocytes (Ls):MM cell cocultures (E:T cell ratio 10:1) in the presence or absence of MM FBs.
Results: Flow cytometry analysis indicated low expression of CD137 on MM NK cells. *In vitro* co-cultures of MM BMLs with MM cells *plus* daratumumab increased the expression of CD137; CD107a and perforin on $CD3^-CD16^+CD56^+$ NK cells suggesting that daratumumab activates NK cells. Furthermore, MP0310 increased adhesion of daratumumab-treated NK cells on MM FBs. Analysis of ADCC in BMLs:MM cells co-cultures in the presence of MM FBs showed that MP0310 enhanced NK cell activity of daratumumab pre-treated BMLs.
Conclusions; MP0310 may represent a new therapeutic strategy to enhance daratumumab-mediated anti-MM activity.

56ASM-0218 | Impact of gestational exercise during maternal obesity in the offspring metabolic-endocrine environment: role of aging

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Background: Obesity prevalence is increasing worldwide, including in women of reproductive-age, rising Maternal Obesity(MO) incidence during gestation. Long-term effects have been described in the MO-offspring(MO-F1), including early development of obesity, diabetes, and other metabolic diseases. Whether gestational exercise(GEx) can rescue MO-F1-effects is still unknown. We evaluated the impact of GEx in preventing MO-F1 metabolic programming through the modulation of circulating metabolic-endocrine regulatory hormones.
Materials and Methods: MO-Sprague-Dawley rat model was obtained by maternal consumption of high-fat/high-sugar diet(HFHS) starting 6-weeks before pregnancy. HFHS-mothers were kept sedentary(HFHS; $n = 5$) or performed GEx(HFHS-Ex; $n = 6$) to compare with the control chow-diet group(C; $n = 7$). Offspring were kept sedentary under a control diet. Blood plasma from male-and female-offspring was collected at 6- 16- and 32-weeks-old. Circulating hormones were quantified using ELISA-kits. T-student or Mann-Whitney statistical-tests were applied(according to data-normality) and $p < 0.05$ was set as statistically significant threshold.
Results: At 6-weeks-old, vaspin plasma levels were affected Circulating vaspin was increased in male-HFHS

and -HFHS-Ex at 16-weeks but decreased at 32-weeks-old. Female-16-weeks-old HFHS-Ex presented lower vaspin concentrations than control and HFHS. Adiponectin circulating levels were significantly increased in MO-F1-16 weeks-old and decreased in 32-weeks-old male and female. MO-effect was rescued in 32-weeks-old HFHS-Ex-females. Leptin levels increase along rat aging being more evident in MO-F1-male. 32-weeks-old leptin levels were partially rescued in HFHS-Ex-male however, exacerbated in females. IGF-1 circulating levels were decreased in 32-weeks male-HFHS and increased in female-HFHS. HFHS-Ex completely or partially-rescued the MO-effect in 32-week-old- male and female offspring, respectively.

Conclusions: MO-programming of endocrine-metabolic axis becomes more evident with offspring aging, and is partially-rescued by GEx. There are sex-specific profiles of circulating adiponectin, leptin, vaspin, and IGF-1, both inconcentrations and behavior, throughout the offspring's time life. It is now essential to understand the molecular and cellular mechanisms behind these regulations and the long-term consequences of the observed alterations.

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56ASM-0270 | Effect of genetic modification by plasmids encoding VEGF and FGF2 on the secretory profile of endothelial cells

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Background: Endothelial cells (EC) cover the inner surface of the heart, blood, and lymph vessels and play an important the regulation of many physiological and pathological processes. The therapeutic effect of endothelial cells has been confirmed in many in vitro and in vivo studies. Oxidative stress, low proliferation rate, poor cell survival and apoptosis after transplantation limit the use of transplanted EC. One of the methods of increasing the therapeutic effect of EC, is the genetic modification of cells.

Materials and Methods: In the current study, human umbilical vein endothelial cells (HUVECs) were transfected with plasmid constructs encoding Vascular endothelial factor (VEGF) and basic fibroblast growth factor (FGF2) and the red fluorescent protein DsRed. 2A-peptide sequences of picornaviruses were used to co-express the genes in a single plasmid vector.

Results: Cultured HUVECs expressed endothelial cell CD markers: CD105; CD54; CD31. Fluorescence microscopy showed that after 24 hours the transfected cells secreted the marker protein DsRed. PCR-RT and ELISA were used to show that transfected HUVECs overexpress VEGF and FGF2 proteins. Cytokine analysis using xMap technology showed that HUVECs secreted high concentrations of PDGF-AB/BB, PDGF-A, G-CSF. GM-CSF, IL-1a, IL-6; RANTES, MCP-3. In turn, high concentrations of chemokines: IL-1a, IL-8; IP-10; MCP-1 were detected in HUVEC cell lysates. Genetic modification of HUVEC with a plasmid containing only the DsRed gene did not affect the secretion of the cytokines studied. Overexpression of VEGF and FGF2 by cells also did not lead to significant changes in the secretory profile of transfected endothelial cells.

Conclusions: Thus, we can assume that genetic modification by these plasmid constructs does not affect the secretory profile of the cells. Overexpression of angiogenic factors VEGF and FGF-2 may increase the anti-apoptotic and angiogenic potential of endothelial cells.

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56ASM-0332 | Sex-specific progression from diastolic dysfunction to HFpEF: rational and preliminary results of the HELPFulUP study.

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Background: Left ventricular diastolic dysfunction (LVDD) can remain pre-clinical but may also worsen over time and deteriorate to symptomatic heart failure with preserved ejection fraction (HFpEF). However, knowledge on the progression from LVDD to HFpEF is scarce. HFpEF is predominantly seen in females, and has a similar poor prognosis as HF with reduced ejection fraction (HFrEF). However, unlike HFrEF, very limited treatment options are available in HFpEF. Preventive strategies

directed towards high-risk individuals with LVDD may therefore be crucial as they might halt LVDD progression to HFpEF. Accordingly, we aimed to describe the sex-specific incidence of HFpEF in individuals with LVDD.

Materials and Methods: Participants seen at a cardiology outpatient clinic and included in the HELPFul study between 2016 and 2019 because of a diagnosis of LVDD (based on a routine echocardiogram) were eligible for this HELPFulUP study (n=213, 60% females). Study procedures include assessment of symptoms and signs suggestive of heart failure, electrocardiography, echocardiography, biobanking and blood measurement of natriuretic peptide levels. Importantly, both rest and exercise echocardiography were performed to classify diastolic function non-invasively as precise as possible including a panel of three cardiologists to (re-)adjudicate LVDD, the study endpoint of HF and the HF phenotype. The Fisher exact test is used to compare NYHA classes and HF incidence between males and females.

Results: Between August 2021 and April 2022 a total of 59 participants (29 women) out of the 213 completed the study measurements and endpoint determination. There were no significant sex differences with respect to NYHA class or functional and structural parameters of diastolic function, with the except of left ventricular mass index, which was higher in males compared to females (83±28 vs. 67±12 g/m2, p=0.01, Table 1). After a median follow-up time of 4.7 (IQR 4.5-4.8) years, seven individuals developed HFpEF, similarly in males and females (10.0 % vs. 13.8%, p=0.56, Figures 1 and 2).

Conclusions: During ~5 year follow-up, preliminary analyses show that 11.9% individuals with pre-clinical LVDD developed HFpEF. Given the small absolute numbers adequate comparison between males and females is yet not possible. At the end of this study we will be able to provide sufficient data to compare males with females, and assess with multivariable logistic regression what determinants are related to progression from LVDD to HFpEF such that it is possible to identify those who are more likely to benefit from early prevention strategies.

56ASM-0217 | Non-immunogenic polymeric nanoparticles as an efficient brain-blood barrier targeting agent

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Background: There are still no available therapies to reactivate brain's function after occurrence of neurodegenerative diseases. There is an urgent need to develop an effective drug carrier capable of crossing the blood-brain barrier (BBB) and promoting the therapeutic brain accumulation of CNS-targeting drugs. The transient opening of the BBB using photothermal energy-generated nanoparticles (NPs) upon near-infrared (NIR) light exposure has gained tremendous interest in the context of brain targeting. It remains elusive if the photothermal energy produced by NPs activates a pro-inflammatory response which might affect the BBB function and integrity. Here we report a polymeric formulation suitable for an efficient BBB crossing without triggering immunotoxicity.

Materials and Methods: We have studied the combined effect of transferrin (Tf) peptide and photothermal energy produced by polydopamine (PDA) NPs on crossing ability through bEnd.3 cells monolayer model.

Results: Our results show the conjugation of 180 Tf peptides per PDA NP (Tf_{180}-PDANPs). Upon NIR light exposure (785nm, 2W/cm^2, 5min), PDA NPs increase the temperature 5ºC. We demonstrate that the photothermal energy generated by PDANPs do not induce M1 macrophage transition and subsequent secretion of cytokines. Importantly, conditioned media obtained from macrophages (exposed to NPs and/or NIR light) do not affect the integrity of BBB model. bEnd.3 cells internalized higher percentage of Tf_{180}-PDA NPs upon NIR light exposure compared to Tf_{180}-PDA NPs/PDA NPs without light exposure. Importantly, both NP and light exposure do not affect the bEnd.3 cells monolayer integrity. The results show that Tf_{180}-PDA NPs cross the monolayer more efficiently than the PDA NPs upon NIR light exposure. Additionally, we show that PDA NPs elicit no acute activation of macrophages after NIR light exposure, causing no immunotoxicity in the BBB model.

Conclusions: Overall, PDA NPs could be used as effective light-responsive carriers to deliver drugs for treating multiple CNS disorders. This work was funded by FCT investigator grant (IF/00539/2015).

56ASM-0327 | Correlation between atrial cardiomyopathy and predictive biomarkers of cryptogenic stroke in primary and secondary prophylaxis: potential role of endothelial progenitor cell (EPCs) dysfunction.

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Recent advances support the concept that pre-persistent Atrial Fibrillation (AF) and paradoxical embolism do not explain the wholeness of embolic strokes, suggesting the recently postulated hypothesis of a broad Atrial Cardiomyopathy (AC). Despite its worldwide distribution, pathogenic mechanisms underlying AC are still largely unknown. Folate cycle disorders are a dysmetabolism only partly explained by methylene tetrahydrofolate reductase (MTHFR)-inherited defects[1]. On a translational basis, folates dysmetabolism could hinder both endothelial and circulating endothelial progenitor cell (EPCs) functioning, therefore providing one-shot explanation to both atrial stasis and endothelial dysfunction, in the context of the Virchow Triade[2]. If such cardiac-bone marrow (BM) networking would be verified, a fundamental pathogenic mechanism of AC and subsequent AF would be unraveled.

Here, we aim to study whether: i) AF patients would show dysfunctional EPCs and ii). atrial fibrosis (AFib - intended as a relative percentage of low voltage area in the context of left atrial endocavitary voltage mapping) would relate to folate cycle disorders (intended as both: MTHFR C677T inherited mutations and BM function disorders, here referring to erythropoiesis diversions)

We studied 59 patients admitted to the Cardiology Unit of the General Hospital "F.Miulli", with preserved EF, subjected to AF ablation and 30 hypertensive patients (as controls), enrolled by the Unit of Internal Medicine and Clinical Oncology, University of Bari Aldo Moro Medical School. AFib was quantified by bipolar peak-to-peak voltage at each acquired point, measured and defined through the relative percentage of low-voltage areas (<0,5 mV) with respect to the wholeness of the picked voltage points. Blood count cell was evaluated at the admission. MTHFR C677T genotypes were elucidated by real-time PCR. Serum folates were measured by a commercial laboratory test. EPCs isolation and characterization were performed by Ficoll-Hypaque gradient and following flow cytometry analysis for cell surface antigens: CD45, CD34, CD133, Vascular Endothelial Growth Factor Receptor2 (VEGFR2) and KDR (Kinase Insert Domain Receptor). EPCs functional wound healing assay was performed to determine the number of EPCs migrated into the "wound", measuring the percentage of relative wound closure compared with matched-controls. In the AF group, number and migration capacity of EPCs was significantly reduced with respect to controls. The AFib percentage significantly differed between C677T MTHFR homozigosis patients (n=15) with respect to non-C677T MTHFR homozygosis patients (n=44). Once univariate analysis was performed, subsequent multivariate analysis highlighted highest fit once merged RBC, RDW-SD and folates values were inputed: goodness of fit was proper, modelling good. Either RBC, RDW-SD and folates coefficient reached significance in AF patients compared to controls.

From data obtained so far, our findings support the hypothesis that genetically determined folates dysmetabolism (MTHFR dysfunction) promotes AFib via a complex cardiac-BM networking involving circulating EPCs and unraveled by erythropoiesis diversions, thereby contributing to AC development.

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56ASM-0096 | A Cellular Senescence Program Iinduced by Extracellular Matrix

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Background: The removal of senescent cells, characterized by growth arrest and apoptosis resistance, by senotherapeutics has proven to be beneficial; however, this process may leave behind senescent extracellular matrix (ECM) that may induce a senescence program in proliferative cells after some time.
Materials and methods: We investigated the composition and mechanical properties of ECM derived from irradiation-induced senescent smooth muscle cells (iSMCs). To understand whether senescent ECM can induce a senescent program in non-senescent cells, decellularized ECM from proliferative SMCs (P-ECM) and iSMCs (S-ECM), was used as a scaffold for the culture of endothelial cells (ECs).
Results: Using vascular cells as a model, we show that ECs cultured in S-ECM, for 2 weeks, initiate a senescence program, in contrast with ECs cultured in P-ECM. Characterization of S-ECM and P-ECM by atomic force microscopy (AFM)measurements showed that the S-ECM presented a higher Young's modulus than P-ECM. Also, proteomics showed significant differences in their protein content, being collagens, proteoglycans and fibulins mostly downregulated, and desmin, vimentin, talin, integrins and cadherins upregulated in S-ECM. Through proteomic analysis, we identified proteins that were overexpressed in S-ECM. Inhibition of a signaling pathway of interest in ECs cultured in S-ECM, decreased the senescence program, with a lower percentage of senescence-associated β-galactosidase (SA-bGal) positive cells and senescence associated genes. Also, gain-loss function studies showed the involvement of a molecular adaptor in the senescence induction effect. Moreover, *in vivo* studies with subcutaneous implantation of disc coated with S-ECM or P-ECM in a young mouse model were performed, and 30 days after implantation, we observed that discs coated with S-ECM also lead to an increase in senescence markers.
Conclusions: To the best of our knowledge, our work demonstrated, for the first time, the effect of the ECM as a senescence trigger and identified a signaling pathway that partially mediates this effect.

56ASM-0288 | Modeling and drug screening on human cardiomyocytes derived from hutchinson-gilford progeria syndrome induced pluripotent stem cells

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Background: Hutchinson-Gilford progeria syndrome (HGPS or progeria) results from a point mutation in the *LMNA* gene, causing the formation of a permanently farnesylated form of lamin A – progerin – that accumulates in the nuclear membrane, ultimately leading to premature ageing. The cardiovascular system is greatly impaired in progeria individuals, causing death usually by myocardial infarction, heart failure, or stroke, at an average age of 14.6 years old. Although the effects of progeria have been widely studied in the vascular system, little is known about its effects on cardiac cells. In addition, it is relatively unknown the effect of clinical and exploratory drugs that have been identified to fight progeria disease, in cardiomyocytes (CM). In the current study we investigate: (i) the impact of progeria in the differentiation of human iPSCs into left ventricle (LV) cardiomyocytes; (ii) the effect of progerin accumulation in the senescence phenotype and metabolism of LV CMs; and (iii) the effect of drugs at the molecular, cellular and functional levels.
Materials and methods: Through WNT signaling pathway modulation, human induced pluripotent stem cells (hiPSCs) derived from dermal fibroblasts of progeria

patients and a CRISPR/Cas9-generated healthy isogenic control line (with a complete correction of the mutation) were differentiated into left ventricular cardiomyocytes, in a 30-day differentiation protocol. On day 30, the CMs were characterized regarding progerin accumulation, senescence-related markers, mitochondrial respiration (resorting to a SeaHorse XF Mitro Stress Assay), and calcium handling (through fluorescence calcium imaging). The effect of a selection of bioactive compounds on these parameters was performed.

Results: Progeria hiPSCs were successfully differentiated into LV CMs. The onset of beating (day 6) and cardiac differentiation efficiency (ca. 75% of CMs) were similar in both progeria-hiPSC and control isogenic lines. In contrast with the isogenic control, progeria-iPSC-CMs expressed progerin, at both gene and protein levels. Notably, progeria-iPSC-CMs exhibited a senescent phenotype, with the gene expression of senescent markers positively correlating with progerin transcript expression. Importantly, results suggest that progeria-iPSC-CMs present changes in mitochondrial respiration and calcium handling. Currently, a group of compounds is being screened as potential treatment strategies to revert the observed phenotype.

Conclusions: In this work, it was successfully generated, for the first time, a reliable *in vitro* model of human HGPS left ventricle cardiomyocytes, potentiating the study of the effects of progeria on patient cardiomyocytes with the possibility of further unveiling insights and treatment strategies for this disease or even, potentially, for physiological cardiac aging itself.

Keywords + Funding: Human induced pluripotent stem cells; Disease modelling; HGPS; Cardiomyocytes.
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SYMPOSIUM 8: MULTIDISCIPLINARY – COLLABORATIVE CLINICAL INVESTIGATION BETWEEN MEDICINE & SURGERY

56ASM-0002 | The value of IL-8 gene polymorphism 845 C/T and Serum Expression of IL-8 in the Endometrial Cancer Risk

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Background: The pathogenesis of endometrial cancer (EC) frequently involves metabolic disorders accompanied by systemic inflammation. Interleukin-8 stimulates inflammation and may increase tumor angiogenesis. Functional polymorphisms in the IL-8 gene promoter region can lead to an imbalance in the chemokine production. The study aimed to assess the levels of IL-8 in serum and neutrophils (Nph), and polymorphism 845 C/T of the IL-8 gene as EC risk factors.

Materials and Methods: The study included 39 patients with stage I-III EC, 10 patients with uterine myoma (comparison group), and 22 healthy women (control group). The level of IL-8 (JSC Vector-Best-Volga, Russia) was determined using ELISA in the Nph lysate and blood serum. To analyze SNP IL-8 845 T/C (rs2227532), restriction fragment length polymorphism PCR was performed using Vsp I endonuclease (SibEnzyme, Russia). The study was approved by the UlSU Ethics Committee. Statistical processing was carried out using Statistica 13.

Results: The non-functional -845C allele was found in 69% of EC patients, more frequently than in the myoma patients (20%, OR 10.1 95% CI 1.9-54.1, $p = 0.004$) and the controls (18%, OR 9.6 95% CI 2.6-34.5, $p < 0.001$). The -845C allele was associated with a decreased production of IL-8 by Nph ($p = 0.00001$). Serum IL-8 levels were higher in myoma ($p = 0.019$) than in the control. In EC, serum IL-8 level did not differ from that in myoma ($p = 0.121$) and were not associated with the genotype. Serum IL-8 level correlated with the age of the EC patients ($r = 0.415$, $p = 0.0003$). The Nph IL-8 content in EC was lower than that in myoma ($p = 0.0001$) and the control ($p = 0.0001$). The IL-8 polymorphism was associated with metabolic syndrome (chi-square 8.5, $p = 0.004$). In the binomial logistic regression, EC was associated with the presence of -845C allele (OR 9.3, 95% CI 1.82-47.6, $p = 0.007$) in combination with metabolic syndrome (OR 9.4 95% CI 1.45-61.3, $p = 0.019$) and older age (OR 1.02 95% CI 1.02-1.15, $p = 0.006$) (Se = 0.87; Spec = 0.88).

Conclusions: Thus, a decreased level of IL-8 in Nph is associated with IL-8 -845C polymorphism. SNP 845-C/T and low levels of IL-8 in Nph, but not in serum, may increase the risk of endometrial cancer, probably through the metabolic syndrome development.

6ASM-0005 | The features of the tumor microenvironment in patients with prostate cancer with different risk progression

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Background: Prostate cancer (PCa) is one of the most common malignant neoplasms among men worldwide. The tumor microenvironment plays a significant role in both carcinogenesis and tumor progression, but the mechanisms of stromal-epithelial relationships and their influence on the course of malignant neoplasms have not been definitively studied to date. *The aim:* to study the features of the PCa microenvironment in patients with different risk progression
Materials and Methods: We studied 60 patients with PCa, who were treated in the National Cancer Institute (Kyiv, Ukraine) in 2015-2017. All patients were thoroughly informed about the study that was approved by the local ethics committee. Evaluation of the components of the stromal microenvironment of tumors was performed using immunohistochemical (expression of immunoregulatory proteins (lactoferrin and osteopontin) and histochemical (collagen (Masson's trichrome staining), mast cells (Toluidine blue staining), *corpora amylacea* (Congo red staining) methods. A morphometric study was performed using the ImageJ program. The levels of immunoregulatory proteins mRNA and miRNA-146a, -214, -221 were assessed using qRT-PCR. Statistical analysis of the results was carried out using the methods of variation statistics using the program GraphPad Prism 8.
Results: Analysis of the results showed that the tumor microenvironment of patients with high-risk PCa progression was characterized by a significantly higher number of mast cells (1.4 times, $p < 0.05$) that often detected between tumor cells (2.0 times; $p < 0.05$) compared with patients with low progression. It was found that in patients with PCa at high risk of progression there is an increase in the amount of collagen (25.0%, $p < 0.05$), and more often (1.4 times, $p < 0.05$) are small *corpora amylacea*, compared to patients with low-risk PCa progression. It was found that patients with high-risk PCa progression are characterized by changes in the ratio of expression levels of immunoregulatory proteins and miRNAs that regulate them compared with patients with low progression.
Conclusions: The obtained data indicate the association of the microenvironment of tumors with the risk of PCa progression and indicate the need for further study of the role of stromal components in the formation of PCa malignancy degree.

56ASM-0017 | CD8+ T lymphocytes infiltrate in mammary tumours chemically-induced in female rats: what is the influence of ketotifen administration?

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Background: Mammary cancer is one of the most frequent cancers among women. Neoplasia are complex masses composed of both neoplastic and non-neoplastic cells, like vascular and lymphatic endothelial cells, adipocytes, mesenchymal cells, fibroblasts, myeloid and inflammatory cells (lymphocytes, neutrophils, eosinophils, macrophages, and mast cells). This work aimed to evaluate the effects of ketotifen on the infiltrate of $CD8^+$ T lymphocytes in mammary tumours chemically-induced in female rats.
Materials and Methods: All experiments were performed in accordance with the legislation on the protection of animals used for scientific purposes. The experiments were approved by the Portuguese Competent Authority (no.008961) and University Ethics Committee (CE_12-2013). Thirty-four female Sprague-Dawley rats were randomly assigned to five experimental groups. At seven weeks of age, mammary tumours' development was induced in animals from groups I, II, III ($n = 10+10+10$) by a single intraperitoneal injection of the carcinogen *N*-methyl-*N*-nitrosourea (MNU). Animals from groups IV and V were injected with saline. Groups II and IV ($n = 2$) were treated with ketotifen in drinking water (1 mg/kg/day, 7 days/week) immediately after MNU administration for 18 weeks, while animals from group III received

the ketotifen only after the development of the first mammary tumour. Groups I and V ($n = 2$) received only water. Animals were sacrificed at 25 weeks of age by an overdose of ketamine and xylazine, followed by an exsanguination by cardiac puncture. Mammary tumors were collected and immersed in 10% buffered formalin. The infiltrate of $CD8^+$ T lymphocytes was assessed by immunohistochemistry using the antibody anti-CD8 (ab33786; Abcam), at a dilution of 1:250, overnight. The immunoexpression was evaluated manually, counting the number of positive cells in five random fields, at a magnification of 400x. Data were statistically analysed using Statistical Package for the Social Sciences (SPSS).

Results: Animals from groups IV and V did not develop any mammary tumor. The iummunoexpression of anti-CD8 was evaluated in 56 tumours (19 from group I, 19 from group II and 18 from group III). The antibody presented a cytoplasmic immunoexpression in all mammary tumours. The mean number of immunopositive cells was 21.75 ± 1.74 in group I, 21.75 ± 1.74 in group II and 22.75 ± 2.01 in group III. No differences were observed among groups ($p > 0.05$).

Conclusions: Apparently, the ketotifen administration did not modulate the infiltrate of CD8+ T lymphocytes in mammary tumours chemically-induced in female rats. Further studies addressing the effects of different concentrations of ketotifen are warranted.

56ASM-0029 | Evaluation of the effectiveness of the motion capture system for indicators of spinal cord injury and its treatment

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Background: Movement analyses became popular in vivo research. So in experimental animal models kinematics can be studied mutually with other biological parameters.

Materials and Methods: Vicon motion capture system was used in kinematics analyses in the case of spinal cord injury in rats. Effect of locally applied self-assembling micellar formulation of methylprednisolone succinate with a trifunctional block copolymer of ethylene oxide and propylene oxide was studied including in terms of functional recovery of kinematics parameters. The obtained data were converted into text format using the ASCII module, after which they were processed using the MATLAB software.

Results: To quantify the amount of motion, the following equation was used to calculate the range of motion $\bar{\varphi}^m = \max_\tau(\bar{\varphi}_+(\tau)) - \min_\tau(\bar{\varphi}_-(\tau))$. When injured, there is no movement of the hind limbs. In the group of rats with spinal cord injury, pronounced steps were absent, motor activity of the hind limbs was not observed, and changes in the angle in the knee joint were mediated by body swaying during the movement of the forelimbs and by the activity of the back muscles. In the group of rats with trauma and methylprednisolone succinate with copolymer, positive changes in locomotor activity were observed compared to rats without therapy. In the group treated with methylprednisolone with polymer, restoration of hindlimb range of motion was observed. The range of motion of the hind limb was 195 ± 25 mm2 ($p < 0.05$).

Conclusions: Our data demonstrate that local delivery of a micellar composition of methylprednisolone succinate after spinal cord injury promotes the restoration of locomotion with body weight support, control of walking direction and balance, as shown by video analysis of movements in the chronic period of injury, that is, we can talk about the neuroprotective effect of the copolymer used. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

56ASM-0031 | The effect of spinal cord stimulation on a rat crural muscle recovery in the period of posthypogravitational readaptation

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Background: One of the most pronounced and rapidly developing effects of functional unloading of skeletal muscles is muscle tissue atrophy. With the resumption of contractile activity, the process of restoring muscle mass occurs slowly, which slows down the recovery of muscle activity, including locomotion. The aim of the study is to evaluate spinal cord stimulation for restoring the weight of the rat crural muscle during readaptation to support load after simulated hypogravity for 35 days.

Materials and Methods: Spinal cord stimulation was performed through pre-implanted electrodes to the S1 segment (RD+ES, $n = 14$). Non-invasive methodical magnetic stimulation was also used (RD+MS, $n = 16$). The following stimulation parameters were used: daily during the readaptation period for 90 minutes in series of 10 minutes with an interval of 10 minutes; the magnitude of the

stimuli is the threshold for contraction of the crural muscle; frequency - 3 Hz. For stimulation effects conducted with data obtained in groups of animals determined under conditions of posthypogravitational readaptation without spinal activation (RD, $n = 18$). Control service data recorded in intact animals ($n = 5$).

Results: It was found that in the RD group, the approximation of experimental indicators to the control of compliance only by 14 days of readaptation: wet weight of SM 91 ± 12% ($p > 0.05$), dry weight was not restored composition 78 ± 13% ($p < 0.05$); wet weight of GM reached 104 ± 8% ($p > 0.05$), dry weight - 89 ± 7% ($p > 0.05$). The most intensive increase in muscle weight is observed during the 3rd and 7th days of the readaptation period. Under the conditions of spinal cord stimulation during the readaptation period (RD+ES, RD+MS), the wet and dry weight of the SM was completely restored by the 7th day of readaptation, and the weight of the GM even slightly exceeded the control values: wet weight 114 ± 10% ($p > 0.05$), dry - 107 ± 6% ($p > 0.05$).

Conclusions: Thus, electrical and magnetic stimulation of the spinal cord show a positive therapeutic effect on the processes of restoration of muscle mass after atrophy due to muscle disuse. The data obtained can be used in the development of effective innovative methods of motor rehabilitation in a number of pathological conditions, in conditions of weightlessness during space flights and during the readaptation period after the resumption of normal/natural conditions for the functioning of neuromotor systems. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

56ASM-0048 | Identification of obesity-related genes-associated proteins in human seminal fluid extracellular vesicles

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Background: Transgenerational epigenetic inheritance (TEI) refers to a gamete-mediated transfer of phenotypic traits not encoded in DNA. We propose that seminal fluid extracellular vesicle (sEVs) cargo responds to metabolic cues and is part of TEI of metabolic disorders. We also propose that spermatozoa can be enriched with obesity-related genes (ORG) (transcripts and proteins), which are carried by sEVs.

Materials and Methods: sEVs were isolated from human seminal fluid, through sequential centrifugation. We isolated two fractions of sEVs. The P100 fraction refers to the sEVs that precipitated at 100.000*.g*. The resulting supernatant was centrifuged at 200.000*.g*. The subsequent sEVs pellet corresponds to the fraction P200. sEVs size distribution was evaluated through nanoparticle tracking analyses. ORG-associated proteins identification was performed by western-blot.

Results: Different populations of sEVs were identified in P100. The 113 nm sEVs population was the most common (1.04×10^6/mL), but populations of 161 nm (7.19×10^5/mL) and 241 nm (1.70×10^5/mL) did also standout. P100 total EVs concentration was 9.86×10^7/mL, and the mean sEVs size was 141 nm. The P200 presented a homogenous EVs population, in which 85nm EVs (6.61×10^6/mL) were the most common. The mean EVs size of this fraction was 112 nm, and the total EVs concentration was 3.48×10^8/mL. The different sEVs populations of P100 and P200 did also carried different ORG proteins. We were able to identify fat obesity gene (FTO) and Melanocortin 4 receptor (MC4R), both associated with sperm quality, in EVs from

P100 and P200. Meanwhile, transmembrane protein 18 (TMEM18) presence resorted only to the vesicles of P100 fraction, but it was not present in the P200 vesicles.

Conclusions: The presence of ORG proteins in sEVs supports the hypothesis that spermatozoa are enriched by sEVs with metabolic cues, such as the ORG proteins. This means that sEVs are potential biomarkers for the identification of a high probability of the offspring inheriting metabolic disorders.

56ASM-0060 | Evaluation of the effectiveness of the motion capture system for indicators of spinal cord injury and its treatment

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Background: Movement analyses became popular in vivo research. So in experimental animal models kinematics can be studied mutually with other biological parameters.

Materials and Methods: Vicon motion capture system was used in kinematics analyses in the case of spinal cord injury in rats. Effect of locally applied self-assembling micellar formulation of methylprednisolone succinate with a trifunctional block copolymer was studied of functional recovery of kinematics parameters. The obtained data were converted into text format using the ASCII module, after which they were processed using the MATLAB software. To quantify the amount of motion, the following equation was used to calculate the range of motion $\bar{\varphi}^{m} = \max_{\tau}(\bar{\varphi}_{+}(\tau)) - \min_{\tau}(\bar{\varphi}_{-}(\tau))$.

Results: In the group of rats with spinal cord injury, pronounced steps were absent, motor activity of the hind limbs was not observed, and changes in the angle in the knee joint were mediated by body swaying during the movement of the forelimbs and by the activity of the back muscles. In the group of rats with trauma and methylprednisolone succinate with copolymer, positive changes in locomotor activity were observed compared to rats without therapy. In the group treated with methylprednisolone with polymer, restoration of hindlimb range of motion was observed. The range of motion of the hind limb was $195 \pm 25\ mm^2$ ($p < 0.05$).

Conclusions: Our data demonstrate that local delivery composition of methylprednisolone succinate with polymer after spinal cord injury promotes the restoration of locomotion with body weight support, control of walking direction and balance, as shown by video analysis of movements in the chronic period of injury, that is, we can talk about the neuroprotective effect of the copolymer used.

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56ASM-0061 | Method of stabilometry data analyses using the empirical mode decomposition and the hilbert-huang spectrum

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Background: Fourier analysis is widespread for stabilometry data. But there are limitations of the method such as linearity and stationarity of the data. The empirical mode decomposition (EMD) offers a potential solution for such limits. EMD is based on 'sift' algorithm and a data-adaptive decomposition that separates a signal into a set of intrinsic mode functions (IMFs). In return, the IMFs permit physically interpretable by Hilbert transforms. The purpose of the study is to develop a method of stabilometry data analyses using EMD and the Hilbert-Huang spectrum.

Materials and Methods: As a result of Hilbert spectral decomposition, the data can be presented in energy-time-frequency terms. To analyze the stabilogram data in the energy-time domain Hilbert spectrum data was reduced by frequencies. Based on the physiological origin of the oscillations the frequency domain was divided into the following intervals: 0 - 0.1 Hz, 0.1 - 0.5, 0.5 - 2 Hz. The Hilbert spectrum data were integrated for each interval by frequency domain. Each spectrum is based on the frequency interval and can be understood as a response to a certain type of feedback. Viz namely there are visual control, vestibular reflexes, and somatosensory activity, respectively to intervals.

Results: Using Fourier analysis there was no significant difference between average amplitudes in all frequency intervals – amplitudes scatter a lot in the frequency intervals. On the opposite, using the proposed method there was a significant difference between average amplitudes in the 1st and 2nd frequency intervals.

Conclusions: Method for reducing the energy-time-frequency domain of Hilbert spectrum to energy-time

is developed. The proposed method allows the automation of the analysis and illustrates the high quality of the received results. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

56ASM-0062 | Influence of legg-calve-perthes disease on the neuromotor apparatus of the lower limbs in children

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Background: Legg-Calve-Perthes disease is a disease that affects preschool and primary school children and leads to early disability. The disease is aseptic necrosis of the femoral head. The aim of our work was to evaluate the electrical activity of the femur and soleus muscles to identify the effectiveness of treatment.
Materials and Methods: During the 10-days treatment course a local anesthetic solution was injected into the lumbar region. 10 healthy boys aged 7 to 9 years old, and 10 boys of the same age with unilateral hip joint disease at III-IV stages of Legg-Calve-Perthes disease were examined in accordance with all ethical standards. The electrical activity of the medial and lateral heads of the quadriceps femoris muscle (QFM) and the soleus muscle of the leg (SM) was recorded at rest and at their maximum voluntary voltage. Then H- and M-responses of SM were recorded during stimulation of the tibial nerve in the popliteal fossa.
Results: In all the examined patients a decrease in the amplitude of the arbitrarily induced electrical activity of QFM and SM was found on the side of the lesion. After the treatment, the parameters of M-responses changed slightly. The thresholds of H-responses of the soleus muscle in patients, compared with healthy ones, were 75% higher before treatment, and their amplitude was 30% lower. After treatment, these indicators actually returned to control values.
Conclusions: Thus, the focus of pathological afferentation located in the hip joint has an inhibitory effect on both the peripheral and central links of the motor apparatus, as evidenced by an increase in thresholds and a decrease in the amplitude of motor and reflex responses in sick patients. The treatment performed improves, mainly, the state of the central link of the neuromotor apparatus.
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56ASM-0068 | Barlow´s test and distraction index in young new zealand white rabbits after hip instability

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Background: The Barlow`s test (BT) and Distraction Index (DI) are medical parameters used in neonates and puppies to assess coxofemoral instability which is linked with later development of hip dysplasia (HD). The rabbit is one of the recommended animal models to study HD. The main purposes of this research were to assess the BT and radiographic hip DI in rabbits after instability surgery.
Materials and Methods: Twenty-one 6-week-old New Zealand white rabbits were used in an HD model and were equally divided into 3 groups: I - control; II - left hip joint instability surgery and right hip joint sham; III - left hip joint instability surgery, followed by hindlimb cast immobilization for 3 days. The surgery was performed to promote joint instability by sectioning the round ligament and allowing the preservation of adequate hindlimbs' functionality. BT and DI were completed after the surgery under general anaesthesia and the hips were allocated into 3 research categories: Normal (N, $n = 21$), Sham Surgery (SS, $n = 7$), and Surgery (Su, $n = 14$).
Results: The BT was positive in all hips of Group-Su and negative in the hips of the remaining groups. A wide DI range was noticed in Group-N (0.21-0.62) and Group-Su (0.44-0.63). The mean DI in Group-Su (0.61 ± 0.12) was higher than in Group-N (0.48 ± 0.11), and Group-SS showed an intermediate mean value (0.52 ± 0.07). The DI differences between Group-N and Su were statistically significant ($P = 0.004$), in the ANOVA Bonferroni Post-hoc Test.
Conclusions: The instability hip surgery revealed a positive BT and an increased DI mean in intervened joints. Therefore, the procedure was effective to promote clinical

and radiographic joint laxity. Nevertheless, the wide DI range observed in normal and intervened joints suggested that some factors, namely a higher development of the pelvic muscle mass in rabbits or the muscle relaxation level under anaesthesia, may be also decisive for the evidence of DI.

56ASM-0069 | The norberg angle in young New Zealand White rabbits after Hip instability surgery

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Background: The Norberg angle (NA) highlights the location of the femoral head concerning the acetabulum and is a worldwide reference parameter in scoring canine hip dysplasia. The rabbit has long been used as an animal model of hip dysplasia. This research had as the main purposes the assessment of the NA in the young rabbits and the evaluation of whether the hip instability surgery interferes with the NA values.
Materials and Methods: Twenty-one 6-week-old New Zealand white rabbits were used in an animal hip dysplasia model and were equally divided into 3 groups: I - control; II - left hip instability surgery and right hip sham surgery; III - left hip instability surgery, following a hindlimb cast immobilization for 3 days. After these medical procedures and under general anaesthesia, the animals were radiographed using the ventrodorsal hip extended view. The NA was then measured and for research purposes, the hips were allocated into 3 categories: Normal (N, $n = 21$), Sham Surgery (SS, $n = 7$), and Surgery (Su, $n = 14$).
Results: The NA ranged from 85º to 101º. The mean ± standard deviation of NA in N, SS, and Su categories was 96.1 ± 3.94, 95.8 ± 2.92, and 96.0 ± 2.44, respectively (ANOVA, $P > 0.05$).
Conclusions: The similarity of the NA values in rabbits with and without hip instability surgery was anticipated and may have occurred due to the effect of the radiographic positioning and the highly developed rabbit's gluteal muscular structure that promoted hip congruence and maintained NA between normal values. The NA mean value for normal hips was lower than the NA of normal hips in dogs (105º). NA also showed a wide range of values. These facts were unexpected and further investigation in adult rabbits and follow up radiographic studies are recommended for a better acknowledge of the normal development of the rabbit hip joint.

56ASM-0071 | Role of microglia and neurons in the maintenance of the perineuronal nets after spinal cord injury

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Background: Perineuronal nets (PNNs) containing chondroitin sulfate proteoglycans and surrounding neurons are associated with increased synaptic stability and decreased plasticity. However, to date, there are no data on PNNs reorganization in the caudal part of the injured spinal cord.
Materials and Methods: We performed immunohistochemistry analysis of ventral horns (VH) at different distances from the epicenter of injury (4, 7, and 11 mm) in the caudal direction at 7 and 30 days after SCI. Antibodies to neuron-glial antigen 2 (NG2), ionized calcium binding adaptor molecule 1 (Iba1), neuronal nuclear protein (NeuN), parvalbumin (PARV), aggrecan (Acan), and choline acetyltransferase (ChAT) were used.
Results: Against the background of no shift in the number of preserved $PARV^+$ neurons in VH near the damage area (4 mm), the number of $PARV^+/Acan^+$ neurons increased at 7 and 30 days, which is also observed at 7- and 11-mm distance. The number of $Acan^+$ neurons decreased at both observation periods and at all studied distances (4, 7 and 11 mm) from the epicenter of SCI. The number of Iba^+ microglia at a distance of 7 mm from the epicenter increased for ~2 times by 7 days and remains elevated by 30 days. The number of $Iba^+/NG2^+$ cells decreased near the damage area (4 mm), which is registered only at day 7 and practically does not change at greater distances from the epicenter at 7 and 30 days.
Conclusions: The results obtained by the project are of practical importance indicating the presence of potential cellular and molecular therapeutic targets in the regions remote from the damage area, which is essential for a more complete restoration of motor function. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by a Presidential Grant for government support of PhD (YM) from the Russian Federation (MK-2737.2021.3).

56ASM-0078 | Study of MicroRNA's profile in the cerebrospinal fluid of patients with spinal cord injury in the acute period

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Background: The aim of the work was to investigate the cerebrospinal fluid (CSF) microRNA profiles of patients with spinal cord injury (SCI) in the acute period and healthy controls.
Materials and Methods: CSF samples were taken by lumbar puncture upon admission of patients to the neurosurgical department No. 2 of the Republican Clinical Hospital (Kazan, Russia). Were studied 2 groups: patients in the acute phase after SCI (day 3) and control group of patients without injury. Libraries were prepared using the SEQuoia Complete Stranded RNA library prep kit. Sequencing was made on the Illumina NextSeq 500. Bioinformatic analysis was performed using the SEQuoia RNA library.
Results: A statistically significant decrease in the expression of 17 miRNAs in the CSF of SCI patients was found when compared with similar samples of the healthy control. The greatest changes were observed for hsa-miR-203a-3p, hsa-miR-145-5p and hsa-miR-130a-3p, their expression decreased in SCI patients (day 3) by 64 times compared with the control group. Targets for hsa-miR-203a-3p include *VSNL* a central nervous system signaling pathway modulator, *LRRTM2* a leucine-rich neuronal transmembrane protein, and *MYEF2* myelin expression factor. Target for hsa-miR-145-5p myelin regulation factor *MYRF*, which is involved in CNS myelination. Targets for hsa-miR-130a-3p, including *RAP2C* - member of the RAS oncogene family, and *ENPP5*, which may play a role in communication between neuronal cells.
Conclusions: We can conclude which miRNAs were affected by SCI and suggest what role they play. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0094 | The effect of spinal cord stimulation on a rat crural muscle recovery in the period of posthypogravitational readaptation

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Background: One of the most pronounced and rapidly developing effects of functional unloading of skeletal muscles is muscle tissue atrophy. The aim of the study is to evaluate spinal cord stimulation for restoring the weight of the rat crural muscle during readaptation to support load after simulated hypogrogravity for 35 days.
Materials and Methods: Electrical (RD+ES, $n = 14$) and magnetic (RD+MS, $n = 16$) spinal cord stimulation was used. For stimulation effects conducted with data obtained in groups of animals determined under conditions of posthypogravitational readaptation without spinal activation (RD, $n = 18$). Control was intact animals ($n = 5$).
Results: It was found that in the RD group, the approximation of experimental indicators to the control of compliance only by 14 days of readaptation: wet weight of SM 91 $\pm$ 12% ($p > 0.05$), dry weight was not restored composition 78 $\pm$ 13% ($p < 0.05$); wet weight of GM reached 104 $\pm$ 8% ($p > 0.05$), dry weight - 89 $\pm$ 7% ($p > 0.05$). Under the conditions of spinal cord stimulation during the readaptation period (RD+ES, RD+MS), the wet and dry weight of the SM was completely restored by the 7th day of readaptation, and the weight of the GM even slightly exceeded the control values: wet weight 114 $\pm$ 10% ($p > 0.05$), dry - 107 $\pm$ 6% ($p > 0.05$).
Conclusions: Thus, electrical and magnetic stimulation of the spinal cord show a positive therapeutic effect on the processes of restoration of muscle mass after atrophy due to muscle disuse. The data obtained can be used in the development of effective innovative methods of motor rehabilitation in a number of pathological conditions, in conditions of weightlessness during space flights and during the readaptation period after the resumption of normal/natural conditions for the functioning of neuromotor systems.
This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

56ASM-0107 | Changing the permeability of mixed bacterial biofilms for antimicrobials

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Background: The formation of a mixed community leads to a change in the metabolic profile of the bacterium, resulting in a change in the biochemical composition of the biofilm. The consequence of such changes may be a decrease or increase in the permeability of biofilms, changing in turn the susceptibility of polymicrobial communities to antimicrobials.
Materials and Methods: Mixed biofilm of *S. aureus – K. pneumoniae*, *S. aureus – E. coli* were obtained. The permeability of mono- and polymicrobial biofilms to various antimicrobials was assessed as described in Anderl, 2000.
Results: Ciprofloxacin was able to penetrate through the extracellular matrix of mono- and polymicrobial biofilms. The penetration of mixed biofilm for ciprofloxacin was significantly higher compared with monomicrobial counterparts. The biofilm of *S. aureus* had the least permeability. On agar plates with *E. coli* zones of growth suppression around all discs did not significantly differ from each other, which is probably due to the high sensitivity of this strain to ciprofloxacin. Moreover around the discs located on the agar plates containing *S. aureus - K. pneumoniae*, an area of partial growth suppression was observed. Growth of both strains was detected in this zone, but the *K. pneumoniae* content was lower. This suggests that both species are capable of survival near the sites of antibiotic exposure, but their metabolic activity changes, while *S. aureus* is more stable under these conditions.
Conclusions: The permeability of the extracellular matrix can change in mixed biofilms. The study of multispecies communities and the analysis of changes in the permeability of their extracellular matrix for drugs can contribute to a more effective selection of antimicrobial therapy.
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56ASM-0111 | Functional role of fibrillar IbpA from phytopathogenic mycoplasma acholeplasma laidlawii

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Background: Small heat shock proteins (sHSPs) are molecular chaperones preventing the irreversible aggregation of cellular proteins. The multichaperone system of *Escherichia coli* includes two homologous sHSPs: fibrillar *Ec*IbpA and globular *Ec*IbpB which in turn competitively interact with the HSP70-HSP100 system. The *Acholeplasma laidlawii* possesses only one sHSP *Al*IbpA which forms a heterogeneous mixture of both globular and fibrillar structures. Here we show the role of fibrillar form of *Al*IbpA in prevention of protein aggregation by chaperone triade sHSP-HSP70-HSP100 in *A. laidlawii*.
Materials and Methods: *Al*IbpA oligomerization was investigated with analytical size-exclusion chromatography and transmission electron microscopy. Protein-protein interaction was studied by bio-layer interferometry and coelution assays. Protein aggregation was assessed by measuring the fluorescence of SYPRO Orange. Chaperone-like activity *in vivo* was evaluated by MTT test.
Results: Regardless of the temperature, a full-length *Al*IbpA forms a heterogeneous mixture of globular and fibrillar structures, while an N-terminally truncated protein (*Al*IbpAΔN12) is present mostly as fibrils. Fibrillar *Al*IbpAΔN12 has higher chaperone-like activity compared to *Al*IbpA and retains fibrillar structure while binding both native and heat-inactivated substrate protein. In contrast, *Al*IbpAΔN12 demonstrates low cooperation with *Al*HSP70 in prevention of proteins aggregation, but does not reduce chaperone-like activity of *Al*HSP100.
Conclusions: The efficiency of the *A. laidlawii* multichaperone system is based on the ability of *Al*IbpA to form both globular quaternary structures, which are necessary for sHSP-substrate dissociation and subsequent protein disaggregation, and fibrils, which, in contrast to *Ec*IbpA, do not inhibit the system *in vivo*. *Al*IbpA can directly transfer the substrate to HSP100 prior to the final disaggregation process, unlike as the hand-over to HSP70, described for *E. coli*, and thereby represents an alternative mechanism in the HSP interaction network. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by RSF grant 20-64-47014.

56ASM-0112 | Evaluation of the synergism of phage therapy with antibacterial drugs in relation to pseudomonas aeruginosa in biofilms

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Background: *P. aeruginosa* causes various infections in immunocompromised individuals. While in biofilm, its treatment became ineffective and contributes to the development of chronic infections, especially in people with cystic fibrosis. The use of phages in combination with antibacterial drugs looks as alternative treatment options. Although the efficiency of phages has been shown in many works, the high strain-specificity of phages requires screening of new ones for development of phage cocktails.
Materials and Methods: *P. aeruginosa* ATCC 27853, 190158 and bacteriophages Ka1, Ka2 isolated from Baikal Lake were used. The minimum inhibitory concentration (MIC) of antimicrobial agents was determined by broth microdilution assay. Cell viability was evaluated in resazurin test. Full-genome sequencing of bacteriophage genomes was performed on the Illumina-SOLEXA (MiSeq).
Results: The Ka1 genome consists of 46092 nucleotides and has maximum identity with the genome of the bacteriophage *Pseudomonas* phage PSA37 (95 % with 79 % coverage), belonging to the family *Podoviridae*. The Ka2 genome consists of 66310 nucleotides and has maximum identity with the genome of the *Pseudomonas* phage S50 (97 % at 99 % coverage), belonging to the family *Myoviridae*. The electron microscopy confirmed that the ultrastructure of Ka1 and Ka2 fits with those of podoviruses and myoviruses, respectively. Ka1 exhibited synergy with amikacin, gentamicin, colistin, meropenem. In combination with bacteriophage, the MIC of amikacin on different test strains was 4-16 times lower, of colistin 16-32 times lower, of meropenem 4 times lower. The bacteriophage Ka2 reduced 4-8-fold the MIC of amikacin, 8-fold of gentamicin, 4-8-fold of Colistin and 4-fold of meropenem.
Conclusions: Ka1 and Ka2 bacteriophages can be used as part of phages cocktails for treatment of infections caused by *P. aeruginosa*.
This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by RFBR grant 20-04-00247.

56ASM-0114 | The first evidence of direct interaction between the bacterial division protein FtsZ and the small heat shock protein IbpA

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Background: In bacterial and eukaryotic cells, small heat shock proteins (sHSPs) are the first line of defense against temperature or oxidative stress preventing the irreversible complete denaturation and aggregation of partially denatured proteins. Eukaryotic homologues of sHSPs have been shown to directly interact with FtsZ, a key cell division protein. Here we show the first evidence of direct interaction between FtsZ and the sHSP IbpA in mycoplasma Acholeplasma laidlawii, that could reflect the molecular mechanism of cell division regulation under stress conditions.
Materials and Methods: The FtsZ-IbpA interaction was investigated by immunoprecipitation followed by MALDI-TOF mass spectrometry. The interaction in vitro was studied by surface plasmon resonance (SPR) and bacterial two-hybrid system. The cellular localization of IbpA and FtsZ in A. laidlawii was assessed using localization microscopy of individual molecules and immunoblotting.
Results: IbpA was observed in FtsZ immunoprecipitates from A. laidlawii cell extracts, which indicates the interaction of these proteins in vivo. The results of SPR and bacterial two-hybrid system confirmed this interaction in vitro. In situ microscopy of A. laidlawii showed the presence of IbpA in aggregates/multimers regardless of temperature. IbpA and FtsZ have a joint localization on the membrane at low temperatures and in the cytoplasm under physiological conditions (30 ºC and 37 ºC) and dissociates completely during heat shock. No effect IbpA on GTPase activity of FtsZ was observed.
Conclusions: We show the first evidence of direct interaction of sHSP with the key division protein FtsZ in prokaryotic cells. The FtsZ-IbpA interaction in A. laidlawii has unknown physiological significance beside the quality control of FtsZ by IbpA and requires further investigations.
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56ASM-0127 | Giant depolarizing potentials during patch-clamp cell-attached recordings in current-clamp mode

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Background: Giant depolarizing potentials (GDPs) are a network activity pattern observed in neonatal rodent and fetal primate hippocampal slices, as well as in the intact rat hippocampus in vitro. Because GDPs are dependent on depolarizing GABA actions, their exploration requires techniques which do not affect intracellular chloride concentrations. Here, we assessed GDPs using cell-attached current-clamp recordings which a priori minimally affect resting membrane potential and intracellular ionic composition.
Materials and Methods: We used concomitant extracellular and cell-attached current-clamp recordings of CA3 neurons form the postnatal days P4-P6 mouse hippocampal slices.
Results: Neuronal network activity in CA3 region of neonatal mouse hippocampal slices was characterized by recurrent GDPs manifested as population bursts and negative LFP deflection in CA3 pyramidal cell layer during extracellular recordings. In the concomitant cell-attached recordings from nearby CA3 pyramidal cells, GDPs were associated with a membrane potential depolarization by 15.6 ± 2.2 mV from the resting potential values of -65.1 ± 9.9 mV. GDPs were also associated with the action potential (AP) firing of CA3 pyramidal cells with on average 4.8 ± 1.3 APs per GDP. APs amplitude reduced at the peak depolarization during GDPs but their depolarization block was never observed. In contrast, during whole-cell recordings with high-chloride pipette solution, APs were suppressed by the depolarization block at the peak of GPDs. Time distribution of APs during GDPs during cell-attached recordings matched population firing during extracellular recordings, whereas in whole-cell mode, neurons mainly fired APs at the ascending and descending GDP phases.
Conclusions: Thus, cell-attached current-clamp patch-clamp recordings are suitable for non-invasive exploration of GDPs and probably of other types of network-driven activity at the cellular level without affecting resting membrane potential and intracellular ionic milieu composition. This work was part of Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030) and funded by subsidy for the state assignment № 0671-2020-0059 in the sphere of scientific activities and RSF 20-75-00055.

56ASM-0134 | Serum concentrations of IL-1RA are increased in obesity and reduced after bariatric surgery in parallel to adiposity

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Background: Excess adiposity contributes to the development of obesity-associated comorbidities such as type 2 diabetes (T2D). Interleukin-1 receptor antagonist (IL-1RA) is a naturally occurring antagonist of the IL-1 receptor with anti-inflammatory properties. The aim of the present study was to compare the circulating concentrations of IL-1RA in subjects with normal weight (NW), obesity with normoglycemia (OB-NG), or obesity with impaired glucose tolerance or T2D (OB-IGT&T2D) and to analyse the effect of changes in body fat percentage (BF%) on IL-1RA levels.
Materials and Methods: Serum concentrations of IL-1RA were measured in individuals with NW ($n = 37$), OB-NG ($n = 75$), or OB-IGT&T2D ($n = 44$), and before and after weight gain ($n = 20$) as well as weight loss (WL) following a dietetic program ($n = 16$) or Roux-en-Y gastric bypass (RYGB) ($n = 63$).
Results: Serum levels of IL-1RA were significantly increased in individuals with obesity, being further increased in the OB-IGT&T2D group (NW 440 ± 316, OB-NG 899 ± 562, OB-IGT&T2D 1265 ± 739 pg/mL; $P < 0.001$). No significant changes were observed in IL-1RA levels which changed 20% from 417 ± 345 to 500 ± 691 pg/mL ($P = 0.647$), after an average period of 20 months in which patients gained an average of 5.3 kg, and their BF% increased 4.2%. In another cohort, after a mean period of 10 months on dietary treatment patients lost an average

of 13.7 kg and their BF% decreased a mean of 8.1%. Diet-induced WL produced a non-significant reduction in circulating IL-1RA concentrations of 14% decreasing from 798 ± 537 to 697 ± 395 pg/mL ($P = 0.368$). Finally, RYGB-induced WL significantly decreased IL-1RA concentrations from 1233 ± 1009 to 660 ± 538 pg/mL ($P < 0.001$).

Conclusions: Serum IL-1RA concentrations are increased in patients with obesity being further elevated in obesity-associated T2D, suggesting the presence of a dysfunctional adipose tissue. WL with marked reductions in BF% is accompanied by a decrease in IL-1RA concentrations, which may reflect the beneficial effects on adipose tissue dysfunction accompanying weight reduction.

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56ASM-0141 | Unravelling the potential fluctuations on antibiotic prescribing tendencies triggered by the COVID-19 pandemic: a countrywide study in Portugal

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Background: The COVID-19 pandemic has rapidly arisen as a public health emergency, resulting in devastating consequences worldwide, particularly within healthcare services. Antibiotics are amongst the most prescribed drugs worldwide, with its inadequate use potentially contributing to antibiotic resistance. This study aims to assess the impact of the COVID-19 pandemic on antibiotic prescribing trends in the context of outpatient care in Portugal.

Materials and Methods: A segmented regression analysis with interrupted time series model was used to evaluate the differences between the monthly prescribed defined daily dose per 1000 inhabitants per day (DID) of antibiotics (those belonging to the Anatomical Therapeutic Chemical (ATC) code J01), as well as of a particular group of antibiotics listed as Watch by the World Health Organization (WHO), between January 2018 and March 2021, in outpatient care, at a national level.

Results: This quasi-experimental study revealed a significant, sharp, and immediate decrease in the overall antibiotic prescribing tendencies promptly upon COVID-19 emergence, but with no statistically significant reduction being noticed over the long term. Moreover, the lowest antibiotic prescribing levels were observed, by far, within the period between April and May 2020, with falls in monthly prescription being higher than 45% in the antibiotics (J01) and over 50% in the antibiotics included in the Watch group.

Conclusions: The findings from this study unveiled the important and significant impact imposed by the unprecedent Covid-19 pandemic on antibiotic prescribing trends in outpatient care in Portugal, namely after the declaration of the state of emergency. The disruption in antibiotic prescription levels uncovered during the current public health emergency may be justified by the resultant home confinement and adoption of preventive measures, which certainly favors a reduction in the incidence of both antibiotic resistances and antibiotic-associated infections.

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56ASM-0151 | A study of vector immunity formation and gene therapy effectiveness based on adeno-associated viruses

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Background: This work aimed to evaluate functional activity and biosafety of AAV9 and AAVrh.10 adeno-associated viruses upon repeated administration to pre-immunized AAV9 pigs. AAV9 and AAVrh.10 encoding cDNA of arylsulfatase-A (*ARSA*) gene intended for gene therapy of metachromatic leukodystrophy were used in this study.

Materials and Methods: Pigs were intravenously injected with AAV9-ARSA ($n = 6$) for immunization by 1×10^{13} GC/kg. 42 days after initial injection, AAV9-coARSA (experimental group 1, $n = 3$) and AAV10-coARSA (experimental group 2, $n = 3$) were intrathecally injected with 1×10^{12} GC/kg. Pigs were euthanized 70 days after AAV intravenous administration. ARSA enzymatic activity was studied in cerebrospinal fluid (CSF) and central nervous system (CNS) samples. ARSA expression

level was determined by qPCR and IHC. ALT, AST, bilirubin, and creatinine-J levels were analyzed in animals' blood serum. Results were compared to a control group of intact pigs ($n = 3$).

Results: ARSA enzymatic activity showed statistically-significant difference between experimental and control groups in CSF and homogenates of pigs' CNS organs. RT-PCR and ICC analyses showed ARSA overexpression in CNS. Compared to AAV9, AAVrh.10 was found to have better ability to transduce spinal cord neurons at the level of cervical enlargement and ganglia of cervical and lumbar spinal cord. AAV9-ARSA repeated administration led to the most efficient neurons transduction of cerebellum (Purkinje cells) and spinal cord's gray matter at the lumbar enlargement level. No statistically significant changes were found in biochemical parameters after drug administration.

Conclusions: Study of vector immunity formation and gene therapy effectiveness based on combinations of AAVs' various serotypes is an urgent task, as it can overcome limitations of viral gene therapy uses. This work was funded by the Russian Government Program of Competitive Growth of Kazan Federal University and the subsidy allocated to Kazan Federal University for state assignment #0671-2020-0058 in the sphere of scientific activities and by Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0152 | Analysis of functionality and biodistribution of AAV/Olig001 encoding arylsulfatase a gene in the porcine nervous system

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Background: Metachromatic leukodystrophy (MLD) is an autosomal recessive disease caused by arylsulfatase A enzyme (ARSA) deficiency, resulting in sulfatides accumulation in neurons and severe neurodegeneration. Adeno-associated virus serotype Olig001 (AAV/Olig001) capsid is known to have predominant tropism for oligodendrocytes' cell surface. Many researchers have shown AAV/Olig001 ability to transduce oligodendrocytes in neonatal mice model of congenital leukodystrophy, that leads to the restoration of a congenital genetic defect. This work aimed to analyze the functionality and biodistribution of AAV/Olig001, encoding arylsulfatase A (*ARSA*) gene and intended for gene therapy of MLD.

Materials and Methods: AAV/Olig001-ARSA containing a unique codon-optimized *ARSA* cDNA sequence was administered intrathecally to pigs ($n = 3$) by $1{\times}10^{12}$ GC/kg. Results were compared to a group of intact animals ($n = 3$). ARSA enzymatic activity was analyzed in cerebrospinal fluid (CSF) every 7 days after drug administration using pNCS (#N7251, Sigma-Aldrich). ARSA expression was analyzed in CNS organs by qPCR and IHC. IHC was performed using monoclonal antibodies to ARSA (#MAG619HU21, Cloud-Clone Corp).

Results: 28 days after drug administration, ARSA enzymatic activity in CSF showed statistically significant increase by approximately 65.2% compared to the point before administration. mRNA of codon-optimized *ARSA* gene was found in the cortex, cerebellum, thoracic and lumbar spinal cord. IHC analysis also confirmed ARSA overexpression in CNS of the experimental group animals.

Conclusions: Therefore, it was shown that following intrathecal administration to pigs, AAV/Olig001-ARSA reaches CNS and leads to ARSA overexpression. Accordingly, AAV/Olig001 can be considered as a promising vector for gene therapy of MLD. This work was funded by the Russian Government Program of Competitive Growth of Kazan Federal University and the subsidy allocated to Kazan Federal University for the state assignment #0671- 2020-0058 in the sphere of scientific activities and by Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0160 | Blood coagulation indexes after COVID-19 in patients with coronary artery disease

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Background: Covid-19 affects not only to the lower respiratory system but also to blood coagulation indexes. Aim of the study was to assess the blood aggregation and coagulation state after Covid-19 in patients with coronary artery disease.

Materials and Methods: 105 patients who were treated in our hospital prior to Covid-19 pandemic and undergone

Covid-19 (mild to moderate severity, after 1-3 months) have been enrolled in this prospective study (aged 43-75 years, mean age 56.8 ± 14.2 years). Baseline characteristics were collected from archival data, and follow-up characteristics were collected when they admitted to the rehabilitation after undergoing Covid-19. Baseline (retrospective) and follow-up anthropometric, laboratory and instrumental data were assessed. All statistical analysis were performed by STATA software.

Results: Blood spontaneous aggregation rate with 0,1 mkmol/L adenosine from 4.3 ± 1.3 to 4.7 ± 1.5%, $P < 0.05$ and with 5 mkmol/L adenosine from 33.2 ± 15.12 to 38.45 ± 17.65%, $P < 0.05$ were increased after Covid-19, however not statistically significant. Blood aggregation speed significantly increased both with 0.1 mkmol/L adenosine from 7.1 ± 2.4 to 9.5 ± 2.5%, $P > 0.05$ and with 5 mkmol/L adenosine from 35.25 ± 14.85 to 44.86 ± 17.60%, $P > 0.05$. Among coagulation indexes fibrinogen (from 382 ± 92 to 585 ± 132 mg/dl, $P < 0.001$) significantly increased after Covid-19 in patients with coronary artery disease whilst prothrombin index, international normalization ratio, thrombin time were not changed significant.

Conclusions: Blood coagulation indexes such as aggregation state and fibrinogen levels are increased after Covid-19 in patients with coronary artery disease. In rehabilitation, one should consider antiplatelet and anticoagulants in patients with coronary artery disease after Covid-19.

56ASM-0179 | Circulating concentrations of IL-36γ and its gene expression levels in visceral adipose tissue are increased in obesity

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Background: Interleukin (IL)-36 is a recently described cytokine with well-known functions in the regulation of multiple inflammatory diseases. The aim of this study was to explore whether levels of IL-36γ are dysregulated in obesity as well as to analyse the effect of weight loss achieved by Roux-en-Y gastric bypass.

Materials and Methods: Plasma IL-36γ was measured in 91 participants [18 lean (LN), 32 with obesity and normoglycemia (OB-NG) and 41 with obesity impaired glucose tolerance or type 2 diabetes (OB-IGT&T2D)]. The effect of weight loss was also evaluated in 31 patients with severe obesity undergoing bariatric surgery. Gene expression levels of *IL36G* and its receptor *IL36R* were analyzed in VAT.

Results: We found increased circulating levels of IL-36γ in both groups of patients with obesity ($P = 0.009$). Circulating levels of IL-36γ were positively associated with the number of leucocytes ($P = 0.026$) and negatively with the percentage of eosinophils ($P = 0.040$). A significant decrease in the circulating concentrations of IL-36g was observed after weight loss achieved by bariatric surgery ($P < 0.001$). Noteworthy, changes in IL-36γ concentrations were positively correlated with differences in triglyceride concentrations ($P = 0.017$) and negatively associated with changes in HDL-cholesterol levels ($P = 0.019$). We showed increased ($P < 0.05$) mRNA expression of *IL36G* and *IL36R* in the VAT from OB-IGT&T2D volunteers, with their gene expression levels being also significantly

associated between them ($P = 0.042$). Gene expression levels of *IL36R* were significantly increased ($P < 0.001$) in the stroma-vascular fraction cells compared to adipocytes while no differences in the expression levels of *IL36G* were found.

Conclusions: Patients with OB and OB-associated T2D exhibited increased circulating levels of IL-36γ and these concentrations decreased after bariatric surgery. Moreover, gene expression levels of *IL36G* and its receptor *IL36R* were increased in the VAT from patients with OB, probably due to infiltrating immune cells.

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56ASM-0180 | OGG1 rs1052133 is a potential predictor of cisplatin resistance in ovarian cancer patients

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Background: 8-oxoguanine DNA glycosylase, encoded by the OGG1 gene, plays a key role in initiating DNA damage repair in the base excision repair. The aim of our research was to assess the role of OGG1 germline SNVs in the development of ovarian cancer (OC) and resistance to platinum-based therapy.

Materials and Methods: FFPE surgical resections and blood of OC patients ($n = 86$) were obtained from Tatarstan Regional Clinical Cancer Center. Patients were divided into platinum resistant (progression during the therapy or within 6 months after) and sensitive (no recurrence in > 6 month after therapy) groups. Control blood samples (H) were obtained from healthy individuals ($n = 36$) with no record of cancer in 3 generations under the KFU ethical committee-approved protocol. Custom targeted DNA NGS panel was designed covering coding region of OGG1.

Results: Four SNVs were discovered in OGG1 gene: rs104893751 (c.137G > A), rs3219012 (c.863C > T), rs3219014 (c.964G > A), and rs1052133 (c.977C > T/G). Rs1052133 resulting in p.Ser326Cys substitution has been associated with an increased risk of chronic myelogenous leukemia, breast, lung, cervical, and colorectal cancers. 326Cys polymorphic variant has a lower ability to repair mutations induced by 8-oxoG. Rs1052133 was analyzed in 42 OC patients received platinum monotherapy to assess its contribution to disease outcome and therapy resistance. GG and CG genotypes were identified in 4.8% and 54.8% of OC samples, respectively. Median time of disease-free survival was 0.756 for GG and 11.441 for CC and CG carriers ($p = 0.009$, Kaplan-Meier Survival Analysis). GG genotype was also associated with advanced FIGO stage ($p = 0.017$, Fisher's exact test), but not with the age of disease onset, cell type, tumor grade or serum Ca-125.

Conclusions: Based on our data rs1052133 might be a promising biomarker for outcome and therapy response prognosis in ovarian cancer.

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56ASM-0194 | eHealthResp online courses: a usability study

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Background: The use of technologies of information and communication in health brings several benefits and significant improvements for health systems. The eHealthResp project comprises the development of two online courses and a mobile app, aiming to support the clinical decision of health professionals in the management of upper respiratory tract infections and promotion of appropriate antibiotic use. The contents of the eHealthResp online courses and mobile app, targeted to pharmacists and physicians, were firstly validated by an experts' panel. This study's main goal is to evaluate the usability of both courses.

Materials and Methods: The usability of the courses, composed of various modules and 4 clinical cases, was evaluated by a group of pharmacists ($n = 6$) and

physicians ($n = 6$), by using the System Usability Scale (SUS). Afterward, descriptive statistical analyses were carried out to assess the usability score, as well as to compare the overall scores provided by both health professional groups. Additional qualitative comments provided were also evaluated.

Results: The average usability score attributed by pharmacists and physicians was of 83.75 (± 15.90, 95%CI) and 78.33 (± 11.57, 95%CI), respectively. Moreover, the qualitative data obtained highlight the user-friendliness of the courses, by presenting good integration of functionalities and strong consistency of contents.

Conclusions: As both quantitative and qualitative feedback obtained were globally positive (in terms of user-friendliness, complexity, and consistency), together with a good usability of the web platform, we believe that the combination of the two courses and mobile app will be useful in clinical practice, as upper respiratory infections are known to be very prevalent diseases.

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56ASM-0199 | How do portuguese health entities communicate with their social media users?

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Background: The large numbers and the rapid spread of social media platforms are transforming not only the forms of communication but also the dissemination of information related to health care. Health entities must be able to adapt and face the challenges created by new communication paradigms and means of engagement with the online public. The aim of this study is to analyze and assess how Portuguese Health Entities use social media platforms to communicate and engage their online users.

Materials and Methods: Data of all the posts created between January and December of 2020 on the social media platforms (Facebook, Instagram, and Twitter) of ten selected health entities were observed by public navigation. The selected indicators of these data were collected in January and February of 2022, through direct observation by researchers.

Results: Facebook was the most used social networking platform by health entities, with the presence of 80% of them. Instagram was used by 50% of the entities, followed by Twitter in which 40% of the entities were present. The type of post most used by all entities on any of their social media platforms was the image. Text publications were the least used by health entities to communicate and disseminate information on their social media platforms. Overall, interaction was higher on image posts, on Facebook and Instagram, while on Twitter it was on video posts.

Conclusions: The results obtained seem to indicate that, although the use of social media platforms and the type of publications have varied among the entities, there are common strategies that deserve attention and should be considered in the creation of guidelines and standard procedures of communication in the social media platforms.

56ASM-0200 | The effect of transcutaneous electrical spinal cord stimulation at a frequency of 20 and 30 Hz on human postural stability

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Background: The effect of transcutaneous electrical spinal cord stimulation (tSCS) on postural stability under conditions of deprivation of afferent inputs of postural control was evaluated.

Materials and Methods: tSCS for 3 minutes was performed at the level of C5 and C6 vertebrae with a frequency of 20 and 30 Hz, stimulus duration 1 ms, stimulus strength 90% of the response threshold in m.Flexor carpi ulnaris, m.Extensor carpi radialis ($n = 6$) in 3 conditions: with open eyes; with closed eyes; on an unstable base. The spectrum of stabilograms was divided into 4 ranges: the range of 0-0.1 Hz, which characterizes visual control, the range of medium-low frequencies - vestibular control, the zone of medium-high frequencies - somatosensory control, high-frequency - proprioceptive regulation. Powers (Pw1, 2, 3, 4) and average amplitude (MeanA1, 2, 3, 4) of each range were analyzed.

Results: In a standard test for tSCS with a frequency of 20 Hz, Pw 4 decreased by 22%; at 30 Hz - by 30% ($p < 0.05$). In the test with visual deprivation during tSCS 30 Hz, Pw4 decreased by 10%, MeanA4 by 21%; Pw3 - by 15%, MeanA3 - by 25% ($p < 0.05$). The decrease in the properties of the support during tSCS with a frequency of 20 Hz increased Pw1 by 71%, decreased Pw2 by 26%; tSCS with a frequency of 30 Hz increased Pw1 by 32% and decreased Pw3 by 14% ($p < 0.05$).

Conclusions: tSCS of the cervical region leads to a change in the contribution of afferent information to the regulation of postural stability. This work was part of Kazan Federal University Strategic Academic Leadership

Program (PRIORITY-2030) and funded by subsidy for the state assignment No. 0671-2020-0059 in the sphere of scientific activities.

56ASM-0203 | FNDC4 and FNDC5 reduce SARS-CoV-2 entry points and spike glycoprotein S1-induced inflammatory cell death in human adipocytes

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Background: The adipose tissue has been proposed as a SARS-CoV-2 reservoir and a cytokine amplification site leading to severe COVID-19 outcomes in obesity. We sought to characterize SARS-CoV-2 entry mechanisms in visceral (VAT) and subcutaneous (SAT) adipose tissue samples of patients with obesity, and whether adipomyokines FNDC4 and FNDC5 modulate SARS-CoV-2 host cell receptors and prevent SARS-CoV-2 spike glycoprotein subunit 1 (S1)-induced inflammatory cell death in adipocytes.
Materials and Methods: Plasma concentrations of FNDC4, FNDC5 and ACE2 were measured in 127 participants, and the expression of SARS-CoV-2 host cell receptors was evaluated in 87 paired biopsies of human VAT and SAT from patients with obesity and normal weight. The effect of FNDC4 and FNDC5 on basal expression of SARS-CoV-2 receptors and on SARS-CoV-2 S1-induced pyroptosis, apoptosis and necroptosis (PANoptosis) was determined in human visceral adipocytes.
Results: Patients with obesity showed increased ($P < 0.05$) circulating ACE2 and an overexpression ($P < 0.05$) of SARS-CoV-2 receptors ACE2, CD147, DPP4 and neuropilin-1 in VAT. Otherwise, low ($P < 0.05$) plasma FNDC4 and FNDC5 levels were found in obesity. Notably, FNDC4 or FNDC5 treatment downregulated ACE2, CD147, DPP4 and neuropilin-1 expression in visceral adipocytes, while *FNDC*4- or *FNDC*5-knockdown in adipocytes upregulated critical genes for SARS-CoV-2 entry (*ACE*2, *DPP*4 and *NRP*1) and priming (*FURIN*). Moreover, co-incubation with FNDC4 or FNDC5 blunted ($P < 0.05$) SARS-CoV-2 S1-triggered NLRP3 inflammasome-induced pyroptosis, apoptosis and MLKL-induced necroptosis in visceral adipocytes.
Conclusions: Together, deranged FNDC4 and FNDC5 in obesity might increase COVID-19 susceptibility due to increased expression of SARS-CoV-2 receptors in VAT, and amplification of SARS-CoV-2 S1-induced inflammatory cell death in visceral adipocytes.
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56ASM-0214 | Trends in prescribing benzodiazepines to older patients: A 3-year nationwide study in Portugal

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Older Patients (> 65 years)
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Background: Benzodiazepines (BZD) are commonly used drugs in older, however, some BZD are considered potentially inappropriate medication (PIM) in this population. Therefore, there is concern about their long-term use since it is responsible for causing drug dependence, cognitive impairment, falls and fractures in older patients. This study aims to analyze trends of BZD prescription to Portuguese older adults in the primary care setting and to analyze the change rate of BZD prescribing over time, assessing the geographical variability between different regions of mainland Portugal.
Materials and Methods: A nationwide, retrospective ecological study was performed between January 2019 and December 2021 for BZD prescribing data reported in a national public database for all persons aged 65 and older in mainland Portugal, from three perspectives: a) The percentage of BZD- Defined-daily dose (DDD)-prescribed in the total of DDD prescribed for all medicines (%); b) DDD per 1000 inhabitants per day (DID) value; and c) BZD-DID change rate (%).

Results: A total of 19 BZD were included in this study. A total of 475 million DDD of BZD were prescribed (3.23 %, of the total of DDD prescribed medicines). The regions North and Center have the higher percentage of DDD of BZD prescriptions (3.95% and 3.63%, respectively) and the South has the lowest number of DDD of BZD prescriptions (2.13%). Alprazolam, lorazepam, diazepam, ethyl loflazepate, and bromazepam were the top 5-BZD that presented the higher DDD values, and between 2019 and 2021, the DID change rate values of these BZD were +7.87%, +5.51%, -1.57%, +5.97%, and +2.90%, respectively.

Conclusions: Considering the results obtained and since Portugal has one of the highest rates of the population over 65 years old in Europe, this study provides relevant knowledge to design new strategies and guidelines to reduce BZD prescription for older adults in primary care.

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56ASM-0231 | The prognostic value of lactate dehydrogenase /albumin to-urea and neutrophil-to-lymphocyte ratios in the prediction of COVID-19 infection associated end organ damage

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Background: Infection by the corona virus strain (COVID-19) and its related syndrome has been associated with more than 6 million deaths worldwide. Early identification of those at risk of progression to critical illness can assist appropriate intensive. We hypothesised differences in biomedical markers and routine haematology indices could provide a potential predictor for COVID-19 induced critical illness and death.

Materials and Methods: This retrospective study included 1488 patient (1139 COVID-19 infection survivors and 349 COVID-19 deaths). Peripheral blood samples were collected from all the study participants and routine laboratory indices, biochemical markers and organ function tests were analysed on admission.

Results: Patients who didn't survive exhibited higher mean corpuscular volume, neutrophil count, neutrophil/lymphocytes ratio (NLR), and lactate dehydrogenase/albumin to urea ratio (LAU) ($p = 0.001$, $p = 0.0013$, $p < 0.001$, $p = 0.0126$ respectively). Results revealed higher infection-related clinical complications shown as higher total bilirubin levels, alkaline phosphatase (ALK), serum creatinine, urea, international normalized ratio (INR), D-dimer, c-reactive protein (CRP), and serum ferritin levels ($p < 0.05$ in all). Lactate dehydrogenase/Albumin ratio was identified as an independent risk factor for end organ damage and clinical complications in COVID-19 positive patients. LAU ratio above 54.7 was significantly and positively correlated with infection-related clinical complications prognostic parameters including INR ($r = 0.171$), D-dimer ($r = 0.176$), serum urea ($r = 0.424$), total bilirubin ($r = 0.107$), ALK ($r = 0.115$), creatinine ($r = 0.365$), CRP ($r = 0.268$), ferritin ($r = 0.385$) and negatively correlated with serum albumin levels ($r = -0.114$) ($p = < 0.05$ in all). Additionally, it had an area under receiver operating characteristic (ROC) of 0.67 (95% confidence interval 0.56 to 0.79) compared to 0.60 (95% confidence interval 0.54 to 0.68) using NLR.

Conclusions: We suggest that combining LAU ratio and NLR in the primary clinical assessment of COVID-19 patients will give a better prediction of people at risk of development of infection-induced end organs damage and thus will help clinicians effectively prioritize intensive interventions to control infection-related complications.

56ASM-0248 | Indocyanine green routine application: a new technology to optimize the outcome of thyroid surgery

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Background: Introduction Total thyroidectomy is associated with high rates of temporary or permanent hypoparathyroidism. During surgery, ICG fluorescence angiography can be used to detect and preserve well vascularised parathyroid glands; this technique has been recently introduced in retrospective and prospective trials as an intraoperative technical support to avoid postoperative hypoparathyroidism.

Materials and Methods: 27 patients undergoing total thyroidectomy were prospectively enrolled in our study. The vascularisation of the parathyroid glands was analysed intraoperatively using ICG tissue angiography. 5mg indocyanine green were intravenously administered. Fluorescence angiography was evaluated in real time

using the PinPoint (Novadaq, Canada) imaging system. The study was approved by the local ethics committee.

Results: CG fluorescence angiography was performed uneventfully in all cases. There was no case of postoperative hypoparathyroidism when at least one parathyroid gland with high fluorescence intensity was preserved. In 4 cases, only low fluorescence intensity was detected in the remaining parathyroid glands after completing the resection. All 4 patients received activated vitamin D3 prophylactically. Two of 4 developed symptomatic hypocalcaemia due to temporary hypoparathyroidism.

Conclusions: Implementation of ICG fluorescence angiography can help in predicting and therefore preventing postoperative hypoparathyroidism after total thyroidectomy. If a well vascularised parathyroid gland with high ICG fluorescence intensity can be secured, calcium substitutionand postoperative prophylaxis of hypoparathyroidism may become obsolete in the future.

56ASM-0250 | How to Better Assess Nodal Metastases Extension during Neck Dissection? The Role of Ultrasound

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Background: Lymph node (LN) metastases from papillary thyroid cancer (PTC) may occur in up to 90% of cases. In case of preoperative or intraoperative clinical or radiological evidence of lateral neck metastases, selective neck dissection (SND) is indicated.

High-resolution cervical US has been reported to be the most sensitive method for detecting nodal metastatic disease. However, it may underestimate actual lateral neck nodal involvement, particularly for lymph-nodes located behind the sternocleidomastoid muscle, where dissection may also potentially increase the risk of postoperative complications. The significance of diagnostic intraoperative ultrasound (IOUS) in metastatic PTC is underinvestigated.

Materials and Methods: We conducted a prospective diagnostic study focused on the use of IOUS in the context of elective SND for PTC by investigating the role of IOUS in detecting metastatic lateral neck lymph nodes from PTC.

Results: We analyzed 33 consecutive patients with preoperative evidence of lateral neck nodal involvement from PTC based on PreUS and fine-needle cytology submitted to surgery between April 2013 and December 2019 at our Academic Unit of General Surgery. IOUS guided the excision of additional nodal compartments that were not predicted by PreUS in nine (27.2%) cases of which eight (24.2%) proved to harbor positive nodes at pathology. The detection of levels IIb and V increased, respectively, from 9% (PreUS) to 21% (IOUS) ($p < 0.0001$) and from 15% to 24% ($p = 0.006$).

Conclusions: In this study has been shown that IOUS has higher sensitivity and specificity than PreUS scans in detecting metastatic lateral cervical nodes. IOUS helped guide the neck dissections, changing the operative strategy in one out of four patients. This study offered evidence that the IOUS may provide accurate intraoperative node mapping, supporting precise SND, and may be of particular value to the units of general or endocrine surgery where radical lateral neck dissection is not routinely performed.

56ASM-0256 | Liver regeneration after partial hepatectomy: new insights combining the p-53 and the gut microbiota in liver's surgical oncology, transplant and in the preoperative ERCP for obstructive jaundice

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Background: The Gut's Microbial Translocation (MT) increases by operative trauma. p-53 and Microbiota considered as the main factors for governing mutations. Our project analyzes their impact on Liver Regeneration (LR) after Partial Hepatectomy (PHx) and in Liver's Surgical Oncology (LSO), Transplant (LT), Preoperative ERCP for obstructive Jaundice (PERCPOJ).

Materials and Methods: (LR) after open (OLPH) and laparoscopic (LLPH) PHx in a healthy porcine experimental model compared, by measuring standard factors with a priority to the post-operative stress and the microbial (Candida species (CS) translocation to the remnant liver (RL). Two pairs of double-series of twenty-nine liver tissues taken from twenty nine porcine livers, after OLPH or LLPH (PD0) and on the 7th postoperative day (PD7), were randomly allocated into two groups: the OLPH (n1 = 19x2) and the LLPH (n2 = 10x2). The liver tissue sections in each group, had been prepared and stained with PCNA, Gaspase-III, anti-Ubiquitin and anti-p-53. PCNA proliferative index (LCPI), Gaspase-III Apoptotic Index (LCAI), and p-53 Index (p-53I). Ubiquitin intracellular stress index (LISI) estimated by microscopic visualization (MV). A PAS-stain CS RL lobule colonization index (CRLLCI) of a sample of twenty eight tissue sections

(n1 = 7x2, n2 = 7x2) while p-53 estimated in a sample of twenty eight tissue sections (n1 = 7x2, n2 = 7x2) by MV.
Results: Indexes compared between OLPH and LLPH on PD0 and on PD7. LISI favored OLPH on PD0 ($p < .1$). LCPI was higher in OLPH on PD7 ($p = .002$). LCANGI favored OLPH on PD7 ($p = .028$). No difference found in the LCAI. No p-53 stained molecules found by MV. CRLLCI was higher in the OLPH in comparison to the LLPH on PD0 ($p = 0.158$) and significantly higher on PD7 ($p = .006$).
Conclusions: Significantly increased surgical, intracellular stress and CS translocation to the RL seems to cause a delay in the LR after OLPH explaining specific clinical implications in LSO, LT, PERCPOJ and the necessity for more personalized approaches to optimize outcomes.

56ASM-0260 | Thyroid fine-needle aspiration cytology: evidence and pitfalls

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Thyroid nodules are very common in the adult population, although the cancer rate among people with a thyroid nodule is low (5%). Moreover, thyroid cancers account for about 1% of all cancer cases, and represents the most frequent endocrine malignancy. In 80–85% of cases the most frequent thyroid cancer is the differentiated thyroid cancer which shows a favorable prognosis while the anaplastic form displays a fatal outcome in approximately all cases. The evaluation of thyroid nodule and the malignancy risk assessment are based on the ultrasound examination followed by a fine-needle aspiration cytology (FNAC) in the case of suspected nodules and lymph nodes. FNAC gives a reliable pre-operative cytological diagnosis with high sensitivity and predictive value, but the main limitations are "indeterminate" nodules or the false negative cytological reports. We will discuss the evidence and pitfalls of pre-operative FNAC and possible remedies to achieve the best accuracy.

56ASM-0265 | Metabolomics and thyroid cancer: a pioneering field

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Metabolomics is a field of growing interest in oncologic research. Although many researchers worldwide have tried to identify the metabolomic fingerprint of thyroid cancer in the last years, bioinformatics issues, physique and diet variability, small sample size, and controls selection limit the quality of the evidence. Building collaborations between different expertise, such as endocrine surgery and molecular biology, helped overcome some of these issues and produced a research protocol based on the metabolomic assay of patients surgically treated for benign and malignant thyroid disease. The paired samples design helped manage the individual variability and selection of controls. In the current protocol, over the next three to five years, up to 400 patients will undergo thyroid surgery (total thyroidectomy or hemithyroidectomy, with or without central or unilateral/bilateral neck dissection) in a single high volume centre for benign or malignant disease. After informed consent, included patients will undergo multiple tissue samplings. Serum samples soon before and one month after surgery will undergo a multi-omics analysis, as will tissue samples of the removed specimens and the possible fine-needle aspiration cytology when available. The aim is to identify a solid signature able to preoperatively differentiate the follicular neoplasms (follicular adenoma versus carcinoma), the different types of papillary cancers according to their risk, and identify aggressive histotypes such as anaplastic cancer. This work explains the details of the protocol and a comprehensive review of the literature on this field.

SYMPOSIUM 9: TRANSITIONAL, TRANSLATIONAL ASPECTS AND GENETICS OF FMF

56ASM-0167 | Clinical features and the course of COVID 19 in family cases of familial mediterranean fever

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Background: Familial Mediterranean Fever (FMF) is a hereditary autoinflammatory disorder characterized by dysfunction of the innate immune system and characterized by recurrent attacks of fever, serositis and synovitis. Colchicine has been the mainstay of treatment for FMF. COVID-19 is not just a simple viral infection; it is an autoinflammatory/autoimmune process that develops as a result of immune system dysfunction. The aim of our study is to determine the clinical and laboratory peculiarities of COVID – 19 infection with FMF and non-FMF members in the same family.
Materials and Methods: 10 FMF patients (7 boys and 3 girls, age from 10 up to 18 years) have been included in our study. FMF children and one of the parents of them (with clinical and genetic conformation of FMF) were drinking the colchicine more than five years. Data regarding diagnosis of COVID -19 infection, clinical presentation, genetic results of FMF, laboratory findings, the results of family tree of FMF patients.
Results: Clinical observations illustrate, that signs of COVID-19 infection in FMF children and one of the parents with FMF had a mild clinical picture of COVID - 19 or without any simple flu-like syndrome despite the epidemic situation. The other non - FMF members of the family had severe clinical signs (high fever, cough, weakness, diarrhea, changes of the smell, muscular pain and pain in the throat) with the high laboratory activity.
Conclusions: According our results and some data of the literature, we hypothesize that colchicine may prevent a severe form of COVID - 19. Prospective, randomized, placebo-controlled studies are needed in this regard.

56ASM-0230 | Aquaporin-3 is upregulated in the monocytes of patients with familial mediterranean fever (FMF)

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Background: Familial Mediterranean Fever (FMF) is an autoinflammatory disease due to mutations of *MEFV* gene which encodes for Pyrin, a protein expressed in white blood cells, mainly neutrophils and monocytes. The Pyrin mutation results in the assembly of the NLRP3 inflammasome, leading to caspase 1-mediated proteolytic activation of the interleukin-1β (IL-1β) and interleukin-18 (IL-18). Aquaporin-3 (AQP3) is a membrane channel facilitating movement of water, glycerol and hydrogen peroxide into and out of cells and whose expression has been also correlated with several inflammatory conditions with suggested involvement in redox signalling, cell migration and NLRP3 activation. Here, we characterize the expression and subcellular localization of AQP3 in human peripheral blood mononuclear cells (PBMCs) and evaluate its expression in monocytes of FMF patients against those of control healthy subjects.
Materials and Methods: BMCs from both FMF patients and control healthy subjects were isolated by density gradient centrifugation using a SepMate™ technology. The isolated PBMCs were used to assess the transcriptional expression of *AQP*3 against that of the housekeeping ß-actin gene and to analyse the extent and distribution of the related protein by confocal immunofluorescence.
Results: By RT-PCR the AQP3 mRNA level was higher in the PBMCs from FMF patients than healthy control subjects. By immunofluorescence, AQP3 was localized at the plasma membrane in the form of punctuated immunolabeling. AQP3 immunoreactivity was much higher in monocytes than lymphocytes. In line with the RT-PCR analysis, AQP3 appeared to be of higher extent in FMF than control monocytes. No difference in AQP3 immunoreactivity was seen between FMF and control lymphocytes.
Conclusions: In human PBMCs, AQP3 is much more expressed in monocytes than lymphocytes. Monocytes of FMF patients show higher levels of AQP3 than control healthy subjects. Work is in progress to see whether the increased expression of AQP3 in FMF monocytes has

correlations with the NLRP3 dysfunction underlying this autoinflammatory disease.

56ASM-0258 | The use of telemedicine in the management of patients with familial mediterranean fever

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Background: Long-term follow-up in rare diseases is critical for adequate clinical management. The pandemic represents a risk period, due to difficult routine workup in referral centers. We therefore aimed to verify the usefulness of telemedicine in a group of patients with Familial Mediterranean Fever (FMF), and to depict an overview of the health status in these subjects

Materials and Methods: FMF patients followed at Clinica Medica "A. Murri", Policlinico of Bari (Italy) were contacted by phone, video-calls and social networks during a 4-months period (March-July 2021). A specific questionnaire was used, and answers were recorded and analyzed.

Results: A total of 51 (20 males) out of 60 patients followed by our FMF outpatient clinic was successfully contacted. Overall, the mean age of symptom onset was 21.1 ± 2.5 years. The mean age of diagnosis was 31.1 ± 2.6 years, with a diagnostic delay of about 10 years. The majority of subjects (57.4%) were on colchicine, which caused mild side effects (mainly diarrhea) in a low number of cases (7/29). The average interval since the last visit was high (46,4 ± 8,2 months). However, the number of FMF attacks since the last visit remained low (0-1) in the majority of patients (74%). The 50% of subjects reported a trigger event preceding the last attack (mainly physical effort, stress, exposure to cold, infections). The 82% of subjects reported as "stable" the health status since last visit. However, a progression towards a more severe clinical course was reported by 9% of subjects, requiring an urgent reassessment.

Conclusions: Telemedicine can be a useful tool in the management of FMF. In the majority of the examined subjects the health status was stable, despite the lack of recent visits. However, telemedicine can efficiently select patients reporting a worsening of the health status, who need an urgent reassessment.

56ASM-0296 | Clinical expression of symptomatic Familiar Mediterranean Fever (FMF) in Lebanese patients: a real-life survey study

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Background: Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disease characterized by recurrent fever and serositis attacks. We aimed to explore clinical aspects of FMF including knowledge, diagnosis, symptoms, and medication in a Lebanese cohort enrolled by *ad-hoc* questionnaire.

Methods: During November 2021-March 2022 we conducted an online survey by a google form questionnaire (33 items) advertised across Lebanese communities, hospitals, internists, and specialists. Patients and children's parents voluntarily provided information about FMF knowledge, diagnosis, presence, and severity of symptoms before and after medication. Since COVID-19 and FMF may share some common symptoms due to activation of the inflammasome pathway, we further investigated this aspect in the FMF cohort with symptomatic COVID-19.

Results: A total of 123 FMF patients participated in this survey (75 females, age range 1-67 years; 10 subjects from Armenia, Persia, and Turkey). The most frequent *MEFV* variants were M694V, M694I, E148Q, V726A, R202Q, and A744S. Before the diagnosis 70% of the subjects had no knowledge about FMF. The diagnosis was late in 40% of subjects (at age ≥20 years). A misdiagnosis occurred in 21% of subjects and was associated with unnecessary procedures such as heavy antibiotic prescription, appendectomy, and abdominal surgery. Prior to the diagnosis and targeted FMF therapy, subjects described typical febrile periodical attacks of systemic serositis with a frequency of more than attack once per month (48%) with intensity ranging from moderate to severe (95%). Following therapy with colchicine, 65% of the subjects reported mild symptoms. In addition, 60% of subjects had COVID-19 infection which was symptomatic in 80% of the cases. Concerning COVID-19, 63% of symptomatic COVID-19 subjects reported that FMF symptoms were higher compared to COVID-19, 23% reported that COVID-19 symptoms were higher than FMF symptoms, and 14% reported no difference between the two diseases. Additionally, 12% reported

consequences of FMF-COVID-19 combined symptoms, mainly joint pain due to persisting arthralgias.

Conclusion: In Lebanon, an endemic region for FMF with a mixture of the ethnic communities from the Mediterranean area, FMF diagnosis can be missed, delayed, or initially erroneously classified. Nevertheless, the diagnostic ability is improving over time. This is the first study in Lebanon to clarify aspects of FMF knowledge, diagnosis, and symptoms as well as evaluation of COVID-19 and FMF interplay. The complex interaction and consequences between COVID-19 infection and the genetic autoinflammatory FMF is being further investigated.

56ASM-0297 | Behçet's disease: a diagnostic challenge in rheumatology

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Behçet's disease (BD) is a chronic multisystem inflammatory disease recently recognized as autoinflammatory disorder [1]. Several pro-inflammatory cytokines, mainly derived from Th1 and Th17 lymphocytes, are involved in different pathogenic pathways leading to development of the clinical manifestations [2]. In this regard, interleukin (IL)-1 seems to play a critical role as it has been found increased both in the serum [3] and synovial fluid [4] of patients with BD. [5,6]. In addition, monocytes from BD patients show an increased expression of the purinergic P2x7 receptor, that is able to promote the inflammasome-driven IL-1β secretion, suggesting the role of innate immunity in BD pathogenesis [7]. Clinically, the disease is marked by the "triple symptom complex", consisting of recurrent oral aphthosis, genital ulcers, and chronic relapsing bilateral uveitis [8], though many other organs, including the vascular, gastrointestinal, and neurological systems as well as the musculoskeletal system can be affected. There are no pathognomonic laboratory tests to diagnose BD, and as such, the diagnosis is based on clinical criteria. More recently several studies have shown that BD patients exhibit increases levels of serum amyloid-A, suggesting a possible role of this inflammatory mediator in the induction of clinical features [9]. Treatment of BD should be tailored according to the extent and severity of clinical manifestations and is aimed at controlling all symptoms, ensuring a good quality of life, and preventing life-threatening complications [10]. The largest experience in BD management has been reached with monoclonal anti-tumor necrosis factor antibodies which have been encouraged for refractory manifestations. Moreover, interleukin-1 inhibitors have proven to be effective as well as safe, despite escalation of their dosage, especially to manage the most severe and difficult-to-treat ocular manifestations [11]. However, general treatment of BD patients remains awkward as protean clinical features may respond differently to the same treatment or even worsen.

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56ASM-0298 | Familial mediterranean fever (FMF) in Apulia: rare, or actually not? A 14 year experience as Internist

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Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disorder characterized by recurrent attacks of fever and serosal inflammation. The clinical features consist of especially abdominal pain, chest pain and arthralgias, plus erysipelas-like manifestations.

According to the available literature, most patients with FMF experience their first attack in early childhood, before the ages of 10 and 20 years in 65 and 90% of cases, respectively. Rarely, the initial attack can occur in individuals older than 50 years of age.

We report our experience with FMF during the last 14 yrs [1], following case #1 aged 36 yrs. [2]. In the regions of Apulia and Basilicata, we could identify several family clusters due to historical and geographical roots. In the initial series of 60 cases, the five most frequent *MEFV* variants were E148Q/R761H (41.9%, compound heterozygosity), K695R (10.2%, heterozygosity), E148Q (8.2%, heterozygosity), E148Q/R761H/A744S (6.1% compound heterozygosity), and P369S (6.1%, heterozygosity). Notably, the mean disease onset was 22 yrs and the diagnostic delay was 15 yrs. The severity of symptoms was generally mild/intermediate but about 30% of this initial series had undergone unnecessary abdominal surgery. Females were significantly older than males (median 40 vs. 30 yrs., respectively, $P = 0.03$). Symptoms including fever were largely responsive to the average dose of colchicine 1 mg/day *ad libitum*. Only one case required canakinumab for resistance/intolerance to colchicine. We did not observe severe cases of secondary amyloidosis and kidney damage. Later, we extended our observations and concluded that the combination of available expert information with sensitive predictor tools could result in a more accurate interpretation of clinical consequences of MEFV gene variants, and a better genetic counselling and patient management, with respect to symptom severity as well [3, 4]. We recently reported the rare case of a very late onset of FMF symptoms in a patient aged 86 [5]. Further studies in FMF have focused the attention on environmental factors including intestinal microbiota [6], COVID-19 pandemic [7], blood-based test for diagnosis and functional subtyping of FMF by the *ex vivo* colchicine assay [8], and histopathological characteristics of synovitis in FMF [9]. Following these seminal observations, we conclude that the Apulia region represents a new endemic area for FMF, a puzzling inherited autoinflammatory disorder. Clinical presentation of FMF can be misleading and requires a complete and early workup to recognize the disease and avoid unjustified surgery. Colchicine remains the gold standard therapy to prevent FMF attacks and fatal long-term complications [10, 11].

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56ASM-0299 | Results of genetic analyses on 225 consecutive FMF patients from the Apulian network on recurrent hereditary fevers

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Familial Mediterranean Fever (FMF), is the most common and thoroughly studied among the hereditary recurrent fever syndromes. Although initially recognized as inherited with an autosomal recessive mechanism, several recent reports have suggested that patients carrying single MEFV variants may present clinical symptoms not different although generally milder compared with patients either homozygotes or compound heterozygotes. Further, the severity of clinical symptoms appears to be partly depending on gene dosage effects, yet to be determined environmental factors, and genetic background including modifier loci. The largest collections of FMF patients so far reported, document genotype-phenotype correlations on rather homogenous populations such as Turks, Israeli, Armenians, Iranians, and Japanese. Italian FMF patients represent a different type of patients' cohort considering that Italians are a rather admixed population due to the rich history of past dominations and being at the center of commercial relationships since centuries.

Here, we present the results of one of the largest collections of Italian FMF patients collected at a single hospital center. 225 different subjects were referred to our unit over a 10 years span. The average age at diagnosis was 23 years. Of the 225 patients, 32 were deemed ineligible for gene testing. The remaining 193 subject were tested with a next generation sequence gene target panel which included the following genes: *MEFV*, *TNFRSFS*1*A*, *MVK*, *NLRP*3 (*NALP*3). In 113/193 tested individuals (58.6%) no variants were identified, while in 80/193 (41.4%) at least one genetic change was detected. The large majority of variants were identified in the *MEFV* gene (67/80, 83.75%) while 5 variants each were present in both *NLRP*3 and *TNFRSFS*1*A* genes and three in *MVK*. Among the *MEFV* variants carriers 21 were simple heterozygous, 38 had two variants, 6 carried three *MEF*V variants and finally there was a single quadruplet. The most significant genotype phenotype correlations will be discussed.

SYMPOSIUM 10: MICROBIOME, METABOLOME AND LIFESTYLES: MORE TO KNOW

56ASM-0040 | Effect of a single bout of exercise on PAHSA lipokine levels in the circulation

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Background: Exercise is an important tool in the prevention and treatment of metabolic disorders. Beneficial effects of exercise are partly based on improving adipose tissue function. Adipose tissue is a source of novel lipokines, i.e. palmitic acid esters of hydroxy fatty acids (PAHSA), which possess potent anti-inflammatory and insulin-sensitizing properties. We have recently shown that regular exercise increases total PAHSA levels in adipose tissue and circulation in humans. However, it is unknown how a single bout of exercise regulates circulating levels of PAHSA and their regioisomers.

Materials and Methods: Therefore, in lean male C57BL/6J mice exposed to treadmill running, we first tested several exercise protocols in terms of achieving the maximum lipolytic stimulus, and then measured plasma PAHSA levels by liquid chromatography/mass spectrometry. Running mice were compared with sedentary animals exposed to a treadmill without running.

Results: Low-intensity treadmill exercise (8 m/min for 40 min, then increasing by 0.5 m/min every 10 min until exhaustion) was the most effective in terms of elevating plasma non-esterified fatty acids and glycerol levels immediately after exercise. This was accompanied by an increase in plasma levels of 9-PAHSA (running, 355.9 ± 35.5; sedentary, 153.2 ± 25.5 pmol/L; $P<0.001$) and 11-PAHSA (running, 72.1 ± 10.5; sedentary, 19.6 ± 5.6 pmol/L; $P<0.01$).

Conclusions: Single bout of exercise is able to raise circulating PAHSA levels in lean mice, but the complete PAHSA profile in the post-exercise period and its dependence on age or obesity remain to be determined. This project was supported by grant No. NU21-01-00469 from the Czech Health Research Council.

56ASM-0051 | Tension-type headache and depression in adolescents

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Background: Tension-type headache (TTH) is a common pain syndrome among children and adolescents. The prevalence increases with the growing up of the child and among schoolchildren TTH occurs in 75%. In children with recurrent TTH, anxiety levels increase with age, and the presence of TTH in childhood increases the risk of depressive disorders in early adulthood.
Materials and Methods: Two groups of patients were examined. Group 1 "Frequent episodic and chronic TTH" (5 or more days with headache per month for 12 months) – 69 patients (15.4 ± 1.4 years), 46.4% boys, 53.6% girls. Group 2 "Infrequent episodic TTH" (no more than 1 episode of headache per month for 12 months) – 61 patients (15.7 ± 1.3 years), 44.3% boys, 55.7% girls. The level of depression was assessed using the Kovac's Children's Depression Inventory adapted and validated into Russian.
Results: A comparison of two groups showed a statistically significant high level of depression in group 1 compared with adolescents in group 2. Statistically significant differences in the level of depression in adolescents with different durations of TTH were revealed ($p<0.05$). The severity of depression according to the Kovac's Children's Depression Inventory in the first group was 69 [67;70], the duration of pain in the same group was 43 [42;47] months, while in the second group the severity of depression according to the same inventory was 21 [20;21], and the duration of the disease was 11 [9;11] months.
Conclusions: Serotonergic dysfunction of the central nervous system is a common pathogenic factor in TTH and depression. Our study showed that depression is a comorbid disorder in children with frequent and chronic TTH. It can be assumed that the lack of timely diagnosis and correction of depressive disorder can be a significant factor in the chronicity of TTH.

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56ASM-0052 | Myogenic etiological factor in tinnitus

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Background: Tinnitus is a borderline clinical situation that is usually treated by neurologists and otorhinolaryngologists. Tinnitus affects 10-15% of adults.
Materials and Methods: 78 patients with complaints of tinnitus were examined. Inclusion criteria: persistent or episodic tinnitus and pain in the head, neck for at least 3 months before study, the presence of at least one active myogenic trigger zone (MTZ) with an area of reflected pain characteristic of the patient. Exclusion criteria: widespread pain; MTZ treatment within 3 months; treatment for tinnitus within 3 months; cognitive impairment that complicates communication with the patient; pulsating tinnitus. 35 patients (55.8 ± 4.5 years) were recruited into the study. MTZ was treated according to the principles of manual medicine.
Results: The modulation of the tinnitus was assessed by the pressure of active MTZ. Decrease of tinnitus was noted in 28 patients with MTZ. The intensity of myogenic pain was assessed by Visual Analogue Scale (VAS): before treatment it was 48.2 ± 2.8 mm, after treatment (10 manual treatment sessions) 18.9 ± 1.9 mm ($p = 0.02$). To assess the intensity of tinnitus, the patients were asked to evaluate this indicator by the VAS (0 – no noise, 100 – the maximum noise that the patient is able to imagine). Before treatment, the noise intensity was 57.9 ± 7.8 mm, after treatment – 20.9 ± 2.5 mm ($p = 0.012$).
Conclusions: In addition to routine neurological and otiatric examination, the examination plan for patients with tinnitus should include: palpation of the muscles of the head and neck for the presence of active MTZ; revealing the modulation of the tinnitus during palpation and pressure of MTZ; treatment of active MTZ.
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56ASM-0066 | PS-test as a tool for cognitive assessment

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Background: Diabetic encephalopathy is a brain damage that occurs in diabetes mellitus as a result of prolonged hyperglycemia with cases of hypoglycemia, combined with insulin resistance of the brain tissue. One of its main complications is cognitive decline. The operational aspects of thinking disrupt mildly, but constantly; patients may lose the ability to distract and generalize. The aim of research is to create a diagnostic tool to reveal cognitive impairment in patients with diabetic encephalopathy at the early stages of its manifestation.
Materials and Methods: We created a specific paremiological subtest (PS-test) which passed linguistic validation in the sample of 300 healthy informants. Then we formed a group of patients (154 males and females over the age of 45 years) with different cognitive complaints. Their cognitive status was assessed using the standard screening neurocognitive MoCA test and PS-test simultaneously. The study was conducted at the University Clinic and at the Center for Speech Pathology at Kazan Federal University.
Results: The correlation of two tests results was revealed: MoCA scores correlate with the number of correctly interpreted paremiological units: MoCA 26-30/PS 8.7; MoCA 22-25/PS 6.9; MoCA 18-21/PS 5.4; MoCA 20 and less/PS 4,2. We identified patients with low PS-test results (from 3 to 6), but MoCA scores were in the range of 26-28, which shows that PS-test is suitable for early diagnosis.
Conclusions: Timely detection of cognitive decline can prevent the development of irreversible complications in patients with diabetes mellitus. The results showed that PS-test can be used in clinical practice in diagnosis of cognitive decline at early stages when standard screening does not show cognitive deficit and the patient has no complaints.
The reported study was funded by Russian Foundation for Basic Research (RFBR) according to the research project 20-312-90044.

56ASM-0106 | Whole genome sequencing and characterization of Limosilactobacillus fermentum AG8 strain producing antimicrobial peptides (AMPs)

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Background: *Limosilactobacillus fermentum* isolated from various sources, including humans, poultry, and pigs, has been shown to be a beneficial probiotic. The use of *L. fermentum* affects body composition and metabolism and has positive effects on human health.
Materials and Methods: *L. fermentum* AG8 isolated from silage was grown for 24 hours at 37°C in MRS broth. Genomic DNA was extracted from the cells using the phenol-chloroform method. A standard Illumina 300 bp paired-end genomic library was prepared from the genomic DNA and sequenced using Illumina MiSeq. 645784 reads were obtained. The quality of the raw sequence data was assessed using the FastQC tool. The graphical reports generated by FastQC were reviewed and the reads were trimmed using Trimmomatic v.0.38.1.0, resulting in 632445 paired-end reads. The trimmed reads were assembled using Unicycler v 0.4.8. Gene predictions and functional annotations were performed using RAST 2.0 (Rapid Annotation using Subsystem Technology). Putative genes and gene clusters responsible for antimicrobial peptide synthesis were predicted using dbAMP 2.0 and CAMPr3 web services.
Results; The draft genome contains 108 contigs (≥500 bp; total length, 4,998,502 bp; N50 length, 199,012 bp; maximum contig length, 714,441 bp; G+C content, 44.9%). A total of 5028 protein code sequences (CDSs) and 113 tRNAs were predicted by RAST. Only 28% of the genetic features were covered by the Subsystems used to annotate genes in RAST. Using prediction tools dbAMP 2.0 and CAMPr3, 3 CDSs with the highest summary prediction score were identified (ILGMBJJF_03519; ILGMBJJF_03557; ILGMBJJF_04150), encoding putative antimicrobial peptides ranging from 55 to 675 amino acids in length.
Conclusions: The availability of the genome sequence of *L. fermentum* AG8 will contribute to a better understanding of the factors responsible for the beneficial properties

of this microorganism and could help in the development of novel probiotics.

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56ASM-0108 | Effect of the PII-like PotN protein on the ATPase activity of PotA subunit of the polyamine ABC transporter in Lentilactobacillus hilgardii cells

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Background: The knowledge of the molecular mechanisms of bacterial adaptation to environmental changes is a basis for understanding of the homeostasis maintaining in human microbiome. The nitrogen/carbon metabolism of most bacteria is regulated by PII-like proteins in response to the availability of energy and nutrition by sensing changes in intracellular levels of ATP, ADP, 2-oxoglutarate and glutamine. In some *Bacilli*, the gene of recently characterized PII-like protein PotN has a unique genetic background and is localized within the *potANBCD* operon encoding the ABC-transporter of spermidine/putrescine, products of amino acid metabolism. Here, we report that PotN protein interacts with the ATPase subunit of the polyamine transporter PotA at increased ADP/ATP ratio and represses is ATPase activity.

Materials and Methods: ATPase activity of the purified C-terminal domain of the PotA protein (PotAc), which performs a regulatory function, has been measured in presence or absence of PotN by the method of Lanzetta et al. adapted by Tom Duncan.

Results: PotAc protein retained ATPase activity and hydrolyzed ATP *in vitro*. The PotN protein itself had no ATPase activity and was unable to hydrolyze ATP. In presence of increased concentrations of PotN, the concentration-dependent loss of ATPase activity of PotAc was observed.

Conclusions: With an increased ATP content in cells, PotN interacts with PotA and reduces its activity. This suggests that the PotN protein acts as an inhibitor of activity of the PotA and consequently of entire transporter, thereby regulating the flow of polyamines into the cell.

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56ASM-0116 Microbiome, Metabolome and Lifestyles: More to Know | Antibiofilm activity of l-borneol possessing 2(5H)- Furanone derivative F131 against S. aureus – C. albicans mixed biofilm

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Background: Biofilm formation appears to be an essential contributing factor that leads to antimicrobial resistance (AMR). Recently, 2(5*H*)-furanone derivatives have been shown to be effective against *S. aureus* and *C. albicans* mono-species biofilms. Considering both the widespread occurrence of *C. albicans–S. aureus* mixed infections, and the previous suggestions to use borneol derivatives as antimicrobial agents, we tested the antimicrobial activity of 2(5*H*)-furanone derivative carrying *l*-borneol group **F131** against *S. aureus- C. albicans* mixed biofilms.

Materials and Methods: MIC and MBC were determined by serial microdilution approach. Using crystal violet staining and drop plate methods, bacterial and fungal viability in mono- and mixed biofilms were assessed in the presence of antimicrobials alone or in combination with **F131**. Synergy of antimicrobials was evaluated in checkerboard assay.

Results: MIC values for **F131** ranged within 8-16 μg/ml for *S. aureus*, and 32-128 μg/ml for *C. albicans*. For biofilms, **F131** completely inhibited the biofilm formation of *S. aureus* at the concentration of 16 μg/ml, and inhibited the *C. albicans* biofilms at 128 μg/ml. Regarding *S. aureus - C. albicans* mixed biofilms, BPC was 128 μg/ml. It could be supposed that antibiofilm activity of **F131** is due to the reduction of viable cells. The synergistic effect of **F131** with conventional antimycotics and antibiotics was tested on mixed biofilms. A synergistic effect was reported with gentamicin-fluconazole mixture and benzalkonium chloride. The presence of 10 μg/ml **F131** was able to significantly reduce the BPCs of both gentamicin-fluconazole mixture and benzalkonium chloride by 4-folds.

Conclusions: Our data shows that the chemotype of **F131** is a promising biofilm-preventing agent, especially, in the course of skin infections caused by Candida -*S. aureus* biofilms. In addition, **F131** increases the efficiency of conventional antimicrobials and thus minimize the required therapeutic doses.

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56ASM-0117 Microbiome, Metabolome and Lifestyles: More to Know | The effect of bovgialuronidase azoximer (Longidaza®) on Candida albicans biofilms

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Background: *Candida albicans* causes various diseases ranging from superficial mycoses to life-threatening systemic infections. Although being a harmless commensal for healthy humans, in immunocompromised patients *Candida* is capable of causing biofilm-associated infections, including mixed fungal-bacterial consortia.
Materials and Methods: The biofilm destruction was assessed by crystal violet, Congo Red staining and scanning electron microscopy. The effect of antibiotics in combination with Longidaze® on the viability of cells placed in the biofilm was evaluated using the MTT assay.
Results: Treatment with Longidaze® led to a 30-60% reduction in biofilm biomass of nine clinical *C. albicans* isolates, and also mixed biofilms of *C. albicans* with different bacteria were destroyed by 30-40%. When fluconazole was co-administered with Longidaze®, the concentration required to similarly reduce the residual respiratory activity of detached cell clumps of three of the four *C. albicans* isolates was reduced by a factor of four. While in the biofilm, two of the four isolates became more susceptible to fluconazole in combination with Longidaza®.
Conclusions: Our data indicate that Longidaza® is capable of suppression of tissues and artificial surfaces biofouling by *C. albicans* biofilms, as well as facilitating drug penetration into the biofilm matrix and cell clumps this way decreasing the effective MIC of antifungals.
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56ASM-0118 | The effect of myrtenol on the S. aureus and C. albicans cell membranes

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Background: The spread of antimicrobial resistance among clinically significant bacteria and fungi is a challenge of 21st century medicine. For natural terpenes and their synthetic analogues, the ability to increase the effectiveness of antimicrobials due to damage to the membranes of microorganisms has been shown.
Materials and Methods: The assessment of the membrane potential of bacteria and fungi after treatment with myrtenol was carried out using the fluorescent dye DioC2. Using fluorescently labeled myrtenol, the rate of penetration of terpenes into the cell was evaluated spectrophotometrically, as well as using confocal laser scanning microscopy.
Results: DioC2 assay showed that in the presence of myrtenol (−) and myrtenol (+), like for the benzalkonium chloride, in *S. aureus* cells the fluorescence intensity decreased in dose-dependent manner, indicating a drop in the cell membrane potential and, apparently, its damage. The fluorescence of *C. albicans* cells treated with myrtenol (−) was comparably with fluconazole. The introduction of myrtenol (+) at maximum concentration led to a significant decrease in fluorescence, indicating serious damage to the cell membrane. The time of half-penetration of the myrtenol into *S. aureus* cells was $t½ = 24 \pm 1.3$ min and $t½ = 26 \pm 1.5$ min for myrtenol (−) and myrtenol (+), respectively. In fungi, time of half-penetration was 18 minutes for myrtenol (+), and 24 for myrtenol (−). CLSM showed that myrtenol (+), like myrtenol (−), penetrates into *S. aureus* and *C. albicans* cells, which confirms our data on the lipophilic nature of interaction with bacterial and fungal cells.
Conclusions: It has been shown that terpenes have a membranotropic mechanism of action on bacterial and fungal cells. Thus, these compounds can serve as promising compounds for complex therapy with antimicrobials to increase their effectiveness.
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56ASM-0175 | Speech and neuropsychological status in a patient with primary early lymphedema of the lower limbs: a case study

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Background: The specificity of cognitive status in patients with lymphedema has not yet been adequately described. We report a patient (female, 51 years old) with primary early lymphedema (PEL) of the lower limbs (stage 3 according to M.Foeldi) complicated by frequent recurrent erysipelas, severe deforming papillomatosis and lymphorrhea, neuropsychological and speech deficit.
Materials and Methods: MRI of the brain; examination and treatment of vascular surgeon, neurologist, nutritionist; neuropsychological (MMSE, MoCA, A.R.Luria's methodology, Schulte tables) and speech (methodology of T.V.Akhutina, L.S.Tsvetkova, N.M.Pylaeva) assessment, cognitive and speech therapy.
Results: A patient was suffering from PEL since 1995 till 2020 without specific treatment. The patient's state steadily worsened, by 2020 she was mostly in bed. In 2021 during treatment at the Center for Lymphology (Kazan Federal University) there were revealed small vessel disease, superior right-sided hemiparesis, pseudobulbar syndrome; exogenous constitutional obesity, arterial hypertension, fatty liver disease, chronic cholecystitis, mild anemia. MRI of the brain revealed moderate focal vascular encephalopathy, degenerative focal changes in the pons, retrocerebellar arachnoid cyst, frontal hyperostosis. The patient underwent complex decongestant therapy, 18 operations (74 papillomas were removed, the weight decreased by 69 kg, the leg circumference decreased to 82 cm). Speech and neuropsychological assessment: moderate pseudobulbar dysarthria; severe acalculia; moderate nonspecific writing disorders; moderate deficiency in executive functions, visual-spatial and kinesthetic gnosis, non-verbal and verbal thinking. After 2.5 months of cognitive and speech rehabilitation there were significant positive changes in visual-spatial and kinesthetic gnosis, non-verbal and verbal thinking, arithmetic skills.
Conclusions: The results show moderately severe cognitive decline (stage 5) according to the Global Deterioration Scale. The effect of rehabilitation is significantly positive. The rehabilitation potential of cognitive sphere is quite high in conditions of systematic neuropsychological and speech therapy.
This work was supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0209 | Analysis of Lap Protein in Monocytes of Patients with Severe Atopic Bronchial Asthma

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Background: In patients with severe asthma phagocytes display reduced apoptosis and phagocytic capability, resulting in accumulated apoptotic cells in the airways. Therefore, a defect in the clearance of dead cells is postulated to underlie the pathogenesis of severe asthma. Increasing evidence has highlighted the role of LC3-associated phagocytosis (LAP) in the regulation of dead cell clearance and inflammation. The aim of this study is to analyze the expression of key LAP proteins in monocytes of patients with severe bronchial asthma.
Materials and Methods: Peripheral blood monocytes from healthy donors and severe asthma patients were used. Monocytes were isolated by centrifugation in an optimized Percoll density gradient. Western blotting was used to analyze Beclin-1, Uvrag, Rubicon and LC3 proteins content.
Results: In the group with severe asthma we observed higher expression of LC3-II form. The process of fusion of lysosomes with autophagosomes can be positively regulated by the UVRAG-Vps34-Beclin 1 complex, as well as be negatively regulated by the Rubicon-UVRAG-Vps34-Beclin-1 complex, i.e. UVRAG and Rubicon proteins are the key regulators. A comparative analysis of these two proteins showed that the expression of the UVRAG protein

was significantly higher than Rubicon. The Beclin-1 plays a key role in the process of autophagy. We showed that the content of Beclin-1 was significantly higher in the monocytes of asthma patients.

Conclusions:This work presents the analysis of some key proteins of the LAP pathway in peripheral blood monocytes of severe asthma patients. Previously, it was found that this group of patients is characterized by cell resistance to apoptosis. Autophagy, an alternative pathway of cell death, is activated in cells. The increased expression of Beclin-1, Uvrag, and Rubicon, established in the present work, allows us to conclude that the LAP pathway is activated in monocytes.

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56ASM-0210 | Effect of smoking on serum pepsinogen values modified by the presence of Helicobacter pylori

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Background: The aim of the study was to investigate pepsinogen values (Pg) in relation to smoking habits and *H. pylori* to further the understanding of the occurrence of false negative cases when using Pg to detect precancerous gastric lesions. In our previous analysis false negatives were associated with current smoking and *H. pylori* status.

Materials and Methods: Serum PgI and II were measured and upper endoscopy with histology was done for participants aged 40-64 within the "Multicentric randomised study of Helicobacter pylori eradication and pepsinogen testing for prevention of gastric cancer mortality: the GISTAR study." Pg values and PgI/II ratio were compared by smoking status, *H. pylori*, gender and age for the general population, additionally stratifying by presence of precancerous gastric lesions.

Results: Of a total of 1210 participants 364 (31%) had precancerous lesions, of which 160 (44%) were false negative. Median PgI and II values were higher among current smokers and those *H. pylori*-positive. *H. pylori* was associated with a larger increase in median PgII than Pg I (110% vs. 42% increase when compared to *H. pylori*-negatives).

For participants with precancerous lesions, *H. pylori*-negative current smokers had a higher median PgI/II ratio than *H. pylori*-positives (3.41 IQR 4.07 ng/mL vs. 1.93 IQR 1.53 ng/mL, $p = 0.02$), but no significant difference in PgI.

Conclusions: The association between current smoking and higher Pg values could play an important role in the occurrence of false negatives in Pg testing for precancerous gastric lesions. *H. pylori* in current smokers seems to counteract the effect of smoking on PgI/II ratio, possibly due to a *H. pylori*-mediated increase in PgII.

56ASM-0211 | Effect of N,N-dimethylacetamide (DMAA) on the viability of cell lines SKOV-3, OVCAR-8 during cryopreservation

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Background: An improvement of technologies for controlling the processes of cell life activity under low-temperature conditions is one of the most important directions in the development of modern medicine and biology. The methods of vitrification allow to preserve the cells structure and the subtle mechanisms of their functioning. Therefore, these methods provide the possibilities to use a tumor cell cryobiobank for monitoring the process of the patient's treatment. This work is aimed at analyzing the effect of cryoprotectants, such as N,N-dimethylacetamide (DMAA), glycerol, dimethyl sulfoxide (DMSO), and a mixture of glycerol-DMAA, on ovarian carcinoma cell lines (SKOV-3, OVCAR-8) by determining the IC50 of these cryopreservatives for these cell lines. The results can be used in the future for the development of cryoprotective mixtures, which will be less toxic.

Purpose of the work: to provide the comparative assessment of the effect of DMAA, glycerol, DMSO and a mixture of DMAA-glycerol solutions on the viability of cell lines SKOV-3, OVCAR-8.

Materials and Methods: The half-maximal inhibitory concentration analysis (IC50) of N,N-dimethylacetamide, glycerol, DMSO and a mixture of DMAA-glycerol was used to determine the amount of solutions , which were necessary to inhibit the biological process by 50% in vitro. The resazurin dye was added to the samples for assessing the viability of cells. The number of surviving cells was assessed on an Infinite 200 PRO multimode microplate reader.

Results: Experimental data show that the concentration of half-maximal cell growth inhibition (IC50) of the mixture of DMAA and glycerol solutions was higher in both cell lines compared to the IC50 of the original solution. Accordingly, cells treated with this mixture had a higher viability.

Conclusions: The results indicate that cryoprotectants, which make cells less susceptible to cryodamage, reduce

the intensity of cell vital processes. A mixture of cryoprotectants DMAA and glycerol has less toxicity than the original DMAA solution.

56ASM-0215 | Executive functions and visual-spatial gnosis in children with sensorimotor alalia and systemic speech underdevelopment

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Background: Sensorimotor alalia (SMA) is a specific disorder of impressive and expressive speech development at the early stages without the previous period of normal speech development according to ICD-10 (usually explained by organic damage of cerebral cortex). Systemic speech underdevelopment (SSU) is a retardation of speech development complicated by other cognitive deficits. It is an urgent question if SSU is more closely connected with the disorders of executive functions and visual-spatial gnosis than SMA.

Materials and Methods: The study included two groups: 44 children with SMA and 56 children with SSU aged 4-8.5. Exclusion criteria: attention deficit/hyperactivity disorder, mental retardation, autistic spectrum disorders, epilepsy, cognitive epileptiform disintegration, pediatric aphasia. All children passed comprehensive examination of speech status and neuropsychological assessment (methodology of T.V.Akhutina et al.). Risk ratios and 95% confidence intervals for unfavorable outcome (UO) were calculated with RevMan5.3 package. UO between SMA and SSU was compared (results were considered significant when $p<0.05$).

Results: Assessment of executive functions in both groups revealed mild deficit: 13 (29%) children with SMA, 19 (33.9%) children with SSU ($p = 0.64$), moderate deficit: 12 (27.2%) SMA, 13 (23.2%) SSU ($p = 0.64$), severe deficit: 5 (11.3%) children with SMA, 14 (25%) children with SSU ($p = 0.1$). Assessment of visual-spatial gnosis revealed mild deficit: 12 (27%) children with SMA, 18 (32.4%) children with SSU ($p = 0.6$), moderate deficit: 7 (15.9%) children with SMA, 12 (24.1%) children with SSU ($p = 0.49$), severe deficit: 3 (6.8%) children with SMA, 7 (12.5%) children with SSU ($p = 0.36$). There was no statistically significant difference between the groups: $p > 0.05$.

Conclusions: The risk of the deficit in the development of executive functions and visual-spatial gnosis (and, consequently, learning disabilities) is almost equal in children with SMA and SSU aged 4-8.5.

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56ASM-0221 | Clinical and electroencephalographic features of neuronal ceroid lipofuscinosis type I: case report

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Background: Santavuori-Haltia syndrome (neuronal ceroid lipofuscinosis type I) is a rare disease with the earliest onset in comparison with other NCL types.

Materials and Methods: The authors analyzed the clinical case of the patient using examination of neurologist, epileptologist, geneticist, ophthalmologist, neuropsychologist, MRI of the brain, video-electroencephalogram (EEG) monitoring; tandem mass spectrometry, molecular genetic analysis by next generation sequencing (NGS), biochemical analyzes.

Results: The female patient (9 years old) with hargoloid dysembryogenesis features and stigmas (flat face, hirsutism, low hair growth, wide nose bridge, epicanthus, half-closed eyelids) had characteristic clinical findings of degenerative disease with regression of all skills until their complete loss in the second year of life, movement disorders in the form of transformation of muscle hypotension into gross spastic tetraparesis in combination with extrapyramidal symptoms, early development of drug-resistant epilepsy with myoclonic, focal and generalized seizures with a tendency to status epilepticus.

The progressive increase in atrophic changes in the brain cerebral hemispheres and cerebellum on brain MRI and negative dynamics on the EEG in - flattening and suppression of cortical rhythmics, the presence of low-amplitude epileptiform activity were of decisive importance in order to suspect a genetic neurodegenerative disease. A homozygous mutation in PPT1 gene previously described as pathogenic, leading to a shift in the reading frame chr1: 40558134A> AT NM_000310.3 c.169dupA p.Met57fs 117 was revealed by NGS.

Conclusions: Further in-depth study of clinical manifestations and electroencephalographic of various forms of NCL is important for the early recognition and development of new treatments for orphan diseases.

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SYMPOSIUM 11: FOCUS ON GENDER MEDICINE

56ASM-0070 | The influence of plant polyphenol resveratrol on human reproductive health

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Background: Natural polyphenol resveratrol (RSV) has been identified as a promising agent in improving human health. This is supported by well-documented evidence regarding its favorable biological properties: antioxidant, anti-inflammatory, anti-obesity, anti-diabetic, anti-ischemic, etc. Summarizing the current data, in this overview we will give data regarding RSV influence on the uterine contractility as well as its role in the diseases such endometriosis, dysmenorrhea, polycystic ovary syndrome (PCOS), embryogenesis and male fertility.

Materials and Methods: The literature search was performed using the NCBI PubMed database. The search covered a period from January 2002 to March 2020 with the key words covering male and female reproductive function and RSV.

Results: The protective effects of RSV are mediated through several mechanisms, including the inhibition of cell proliferation, induction of apoptosis, reduction of inflammation and damage elicited by ROS as well as smooth muscle relaxation. However, the different study designs, as well as the large variability in the dose, route and courses of RSV administration, make it difficult to reach strong conclusions about the health benefits of RSV as a phytoestrogen. Also, recent data are addressing the importance of the phase of a menstrual cycle in which RSV supplementation commences. Its restriction during the proliferative phase may promote ovarian function without further affecting embryo implantation. In contrast, RSV treatment during the initial phase may inhibit decidua transformation of the endometrium. The positive effect of RSV on the prevention of pregnancy-related complications has been observed in both pre-clinical and clinical studies. One of the examples of the beneficial effects is in the treatment of diabetic complications in pregnancy as well as relaxation of umbilical blood vessels, reducing the risk of preeclampsia.

Conclusions: It seems that RSV is a promising compound, can prevent or slow the progression of endometriosis, dysmenorrhea, PCOS and their symptoms. However, there are limitations regarding its bioavailability and pharmacokinetics.

56ASM-0077 | Gender characteristics of hemodynamic parameters in adolescents

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Background: The study of risk factors for the development of the cardiovascular system in adolescents, taking into account gender, is an important scientific direction. The aim was to study the indicators of central hemodynamics in adolescents.

Materials and Methods: Indicators of the functional state of the cardiovascular system were taken from 340 adolescents of different sexes of the 5th, 7th and 9th grades

of Kazan with a two-channel 4-electrode reopletismograph using an attachment for computer analysis with an active change in body position.

Results: In the 9th grade, there is a high degree of correlation in girls ($r = 0.86$) for blood pressure, heart rate ($r = 0.68$) and a negative one for stroke volume ($r = 0.69$). That is, the more hours of the weekly training load, the lower the stroke volume of blood. In girls of all studied classes, there is a high degree of correlation in terms of the IOC ($r = 0.79$) and TPVR ($r = 0.58$). According to the indicator of specific peripheral resistance and the total training load, connections were determined in all classes in girls ($r = 0.72$), and in boys in all classes in a sitting and standing position ($r = 0.81$). We recorded a high feedback in terms of the heart index ($r = 0.89$ and $r = 0.93$) in boys in the sitting and standing position, and in girls in all the classes studied, this indicator is lower ($r = 0.76$ and $r = 0\ .57$).

Conclusions: Gender features become more important in the 9th grade, a high relationship between the studied parameters of the cardiovascular system and the academic load is preserved in girls and is not manifested in boys.

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56ASM-0081 | Gender differences, behavioral characteristics in the lifestyle of modern adolescents and the impact on their health

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Background: In modern conditions, the way of life determines the formation of the health of boys and girls, to improve the system for ensuring optimal development. The goal was to study the lifestyle and behavioral characteristics, taking into account the gender characteristics of adolescents.

Materials and Methods: The method for studying the way of life used the method of anonymous questioning. We interviewed about 458 respondents aged 15-18 years.

Results: The results of our research showed that 64.7% of young men consider it necessary to adhere to the principles of a healthy lifestyle, while girls - only 44.9% of respondents. There was a positive relationship between visits, the duration of additional classes and the presence of pain in the stomach ($r = 0.22$), the frequency of their visits and headache ($r = 0.45$), dizziness ($r = 0.43$), difficulty falling asleep ($r = 0.55$), back pain ($r = 0.56$) and bad mood ($r = 0.78$). At the age of 15 in girls, the later they get up, the less headaches ($r = 0.25$), while in boys, the earlier they go to bed, the less headaches ($r = 0.29$). The later the girls go to bed, the more often they are irritable ($r = 0.31$).A negative relationship was established between girls' sports activities and headache ($r = -0.47$). Sports activities reduce the prevalence of headache ($r = 0.45$) and back pain ($r = 0.38$) in young men as well. Young men perceive and understand health taking into account as a whole, then the girls are "disease-oriented". It is important to take into account and take into account when conducting, developing programs of health-improving activities.

Conclusions: Thus, during the entire period of study, the deterioration of health is more pronounced in girls (63.4%). However, it can be assumed that the significance of gender differences can be offset by the impact of socio-economic factors. "This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030)".

56ASM-0125 | Eating behaviour and morbidity of children with digestive organs diseases (Gender Aspect)

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Background: Early onset of the digestive organs' diseases (DOD) in children under 14 years old is specified by peculiarities of their clinical picture, gender composition, untimely revealing of pathology and results in chronicity of diseases. Aim: To analyze DOD morbidity and assess the dietary pattern and habits of children aged 10-14 years.

Materials and Methods: The analysis of morbidity was carried to the data of the Report Form N 030-PE/o-12 "Data on preventive medical examinations of minors". Nutritional assessment of 200 children was performed based on a questionnaire including 45 questions on the child lifestyle, dietary pattern and formation of food preferences.
Results: According to the medical examination data in the age category 10-14 years, DOD is observed in 29.8% of boys, and in 39.3% of girls. Prevalence of DOD in aged up to 14 years is characterized by negative trend $R^2 = 0.6218$ and a growth rate of 224.9% for the years of 2010–2018. Statistical significance of DOD prevalence between coeval boys and girls made $p < 0.05$ at the age of 10-11; and $p < 0.01$ at the age of 12-14. Risks of gastritis in girls aged 10-14 years (OR1 = 6.7 ± 0.49, OR2 = 6.5 ± 0.54) and those of duodenitis (OR1 = 8.0 ± 0.49, OR2 = 8.4 ± 0.54) were significantly higher. In girls (82 %), there was predomination of refined foods (sugar, high-grade flour, white rice, and etc.) in their diet. In boys (73. %), there was deficiency in fiber intake (vegetables, fruit, greens) in the diet, which resulted in malfunctioning of normal intestinal activity.
Conclusions: Girls aged 10-14 years are more susceptible to DOD. Imbalance and diet violation in periods of the most intensive morphofunctional changes in the child body belong to the group of modifiable risk factors of DOD. This paper has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

56ASM-0201 | Impact of biological sex and aging on the metabolic effects of high-fat diet in mice

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Background: Age and biological sex potentially influence metabolic homeostasis. Age-related adipose dysfunction is a major cause for a high risk of obesity and its associated comorbidities. Males have greater risks of developing these pathologies than females. Our objective was to study the effect of sex and aging in energy homeostasis.
Materials and Methods: Male and female C57BL/6J mice were fed *ad libitum* a normal chow diet or a HFD for 12 or 32 weeks (n = 7-10 per group). Body weight and food intake were registered twice weekly. At 12 and 32 weeks of age, mice were sacrificed. Glucose tolerance test, serum concentrations of adiponectin, insulin, free fatty acids (FFA), triglycerides (TG) and cholesterol were measured.
Results: Body weight, body weight gain and adiposity index at 32 weeks of age in both sexes were increased with HFD, but HFD-fed female rodents showed these changes in a significantly lower extent than males, despite exhibiting increased relative food intake. These differences were not observed at 12 weeks of age. Basal glucose levels at 32 weeks of age were also increased after HFD, reaching significant differences in male mice at both ages. The glucose area under the curve was significantly higher in males fed normal and HFD at 12 and 32 weeks of age than that of female mice in the same conditions. Furthermore, male mice showed lower plasma adiponectin and higher insulin levels than females at 12 weeks of age. Finally, FFA levels were similar in HFD fed females and males, with males exhibiting increased levels of cholesterol and TG as compared to females at both ages.
Conclusions: Aging and sex differences are consistently emerging as important factors in the development of obesity and insulin resistance, factors that are primary contributors of metabolic syndrome. Glucose and lipid metabolism are specifically controlled in males and females. The observed sex and aging-related differentiation may provide an explanation for the protection of the development of obesity and its related diseases in females.

56ASM-0251 | Family care, employment and health of women: An international comparative view

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The multidimensional pressure of simultaneous family care responsibilities, employment outcomes and health dynamics enact multiple effects on individuals, especially on women. In fact, women in Europe are traditionally the major suppliers of family care. This results in a lower presence of women in the labour market (employment) compared to men, as these latter bear lower family burdens. The unequal share of family care responsibilities between men and women also influences health perceptions and outcomes. We wonder if this is more a cause rather than an effect for women having multiple roles (housework, childcare, care of nonautonomous family members, like elderly and disabled people). In this contribution, we investigate the role of family responsibilities in shaping employment and health outcomes, in an international comparative view, by considering a wide time period. We use cross-sectional data from the European Union Statistics on Income and

Living Conditions (EU-SILC) survey for (many) European Countries. Our empirical strategy allows us assessing the effect of family care responsibilities on both employment probability and health outcomes. The models are estimated separately by gender on the time span from 2005 to 2020. This enables us to ascertain the possible effects of the Great Recession and subsequent austerity measures, and the current COVID-19 pandemic on the role of family care responsibilities on employment and health by gender. Our results suggest a disadvantage for women, since most family responsibilities, i.e. care of children, elderly and/or disabled household members, are mainly negatively associated with their employment and health outcomes.

56ASM-0252 | Gender roles and fertility intentions in Italy

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Recent studies have paid great attention to the relationship between couples' gender role-set and fertility. If across developed countries fertility declined after women's massive entry into the labor market, then there has been a reversal of this link, and the last decades' fertility has been higher in those countries where women employment rate is higher. These differentials have been explained with the theory of Gender Revolution (GR), which focuses on the change from the male-breadwinner model to new egalitarian forms based on dual-earner couples (Goldscheider et al. 2015; Esping-Andersen 2009; Esping-Andersen & Billari 2015). On one hand, the progressive adaptation to job activity of women in some countries has resulted in more egalitarian societies and in more egalitarian couple role-set, which permit to women to re-conciliate work and family. On the other hand, the GR theory is predicting negative effects on fertility of the possible inconsistency between gender equity – i.e. the perceived fairness of gender roles – the actual gender equality (McDonald 2000, 2013). This seems the case of Italy, where women are progressively higher educated, but they work in a context of limited childcare services and they still bear the bulk of household chores, even in dual-earner couples.
In this paper we use the most recent survey data from the 2016 Family, Social Subjects and Life Cycle (FSS) to test the GR theory by examining whether the unequal division of housework leads to lower fertility intentions. Our analyses look at couples, with or without children, aged 20- 45, resulting in a sample of 3231 cases. We also build composite indexes of housework burden. Our results confirm that positive fertility intentions diminish with the domestic burden of women, but also give some interesting insights when distinguishing by parity, either by the number and the gender of children of the couple.

56ASM-0253 | Mental and physical health in patients with major psychiatric disorders: does gender matter?

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Background: Pathogenesis of Major Psychiatric Disorders (MPDs), including Major Depression Disorder, Schizophrenia and Bipolar Disorder involves a complex interaction between environmental and genetic risk factors, including socio-economic and life-style variables along with genetic variation distributed along the whole genome. This complex interaction is likely implicated in both physical and psychopathological aspects of mental health. Furthermore, while evidence reports that the clinical phenotype of MPDs is affected by gender differences, it is possible that the same gene-by-environment interaction is implicated in gender-sensitivity to both clinical outcomes and physical side-effects of psychotropic treatments.
Materials and Methods: Here we will present a review of literature, supporting evidence of a biological by psychosocial factor interplay in the pathogenesis of gender specific aspects of mental health, along with data by our group on gender-specific aspects of metabolic dysfunction induced by psychotropic medication.
Results: We will illustrate how different molecular pathways related to neurotransmission and hormone system, supported by as many genetic and genomic risk factors, may crosstalk with complex social variables on determining gender specific physical and mental phenotypes of MPDs.
Conclusions: We will emphasize the overall lack of research on the topic of gender and mental health, showing how disentangling the intricate interaction between gender-biology and gender sensitivity to environment in physical and mental correlates of these disorders can importantly contribute to our overall understanding of these disorders' pathophysiology.

56ASM-0254 | Long covid and gender differences: implications for return to work

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Long COVID syndrome has gained wide recognition in the scientific and medical communities as it widely affects survivors of COVID-19 at all ages and regardless of disease severity levels. Long COVID, however, remains poorly understood, and the predisposing characteristics of patients, the duration of symptoms, and possible risk factors for their persistence are all factors that need further clarification. Although no definitive conclusions have been reached, several studies have specifically investigated potential sex differences, showing that females are more likely to develop post-COVID-19 symptoms than males. Several underlying mechanisms could explain the higher prevalence of post-COVID in females: Differences in the expression of angiotensin-converting enzyme-2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) receptors, immunological differences, such as lower production of pro-inflammatory interleukin-6 (IL-6) after viral infection in females, role of hormones in perpetuating the hyperinflammatory state of the acute phase even after recovery, stronger production of IgG antibodies in females in the early phase of the disease. Finally, increased psychological stress may also play a role in the development of post-COVID symptoms. Prolonged COVID symptoms may further hinder women, affecting their work life and ability to return to work. This situation is likely to be much more challenging for pre- and post-menopausal women, considering that many of the symptoms of Long COVID (fatigue, muscle pain, palpitations, cognitive impairment, sleep disturbance) overlap significantly in the two conditions. Strategies promoting return to work for working women will have to be implemented and could be similar to programmes developed for other chronic conditions, allowing a reduced risk of long-term disability. Further clinical investigations should be carried out from a gender perspective to clarify the interaction between Long-COVID and work, considering the highly gendered nature of work, particularly in female-dominated sectors such as health and social care.

56ASM-0268 | Gender differences in cardiovascular diseases

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There is a wide range of evidence that gender-related differences are present in cardiovascular diseases (CVDs). Women have peculiar characteristics in cardiac structure and function, specific sex hormones, and psycho-social profile that could promote susceptibility to CVDs. This risk in women is often underestimated due to false perception that women are more "protected" than man against CVDs, although CVDs represent one of the main causes of mortality for women. The lower risk of CVDs in premenopausal women could be attributed to protective role of estrogen on eNOS, lipid profile and blood pressure.
The classic risk factors for CVDs are the same in women and men, but there are differences in the prevalence and severity of general risk factors (i.e., hypertension, dyslipidemia, diabetes mellitus, smoking, obesity, physical inactivity) and women-specific risk factors (hypertensive disorders of pregnancy, autoimmune disease, depression and psychosocial stress). Several mechanisms are responsible for the gender differences as well as for the clinical manifestation and outcomes of CVDs.
The present review will focus on the current knowledge about the sex-associated biological differences and gender-dependent sociocultural issues in CVDs. There is an urgent need for more personalized clinical medicine approaches to achieve an ameliorated quality of life of patients and better outcomes in population health.

56ASM-0275 | Insulin and glucose metabolism-based clustering analysis discloses different lipidomic profile among women and men

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Background: Lipids have a relevant role in the development and progression of T2D. Through the regulation of intracellular pathways, lipids can act as signaling molecules and impact insulin action and metabolism. It

is, however, known that there are gender differences in lipid metabolism that will then affect glucose metabolism. Herein, we hypothesized that subjects stratified according to glucose metabolism-related parameters have a different but gender-dependent lipidomic profile.

Materials and Methods: We performed hierarchical clustering analysis based on fasting insulin secretion ($_f$ISR), clearance ($_f$IC), and resistance (HOMA-IR) and insulinogenic index (IGI) during OGTT of 953 subjects from the PREVADIAB2 cohort (subjects with normoglycemia, prediabetes or diabetes). The resulting clusters were profiled according to insulin secretion and resistance, and the lipidome of 273 females and 215 males was assessed by LC/MS-QTOF.

Results: The four identified clusters were named according to their main metabolic features: Liver Sensitive (LS), Pancreas Glucose Sensitive (PGS), Insulin Deficient (ID), and Insulin Resistant (IR). The LS cluster had the most advantageous lipid profile, whereas the other clusters presented lipid and/or glucose dysmetabolism. Although the pattern of glucose metabolism-related parameters was similar in men and women among clusters, the lipidomic profiles were different. Globally, women presented higher sphingomyelins and lower lysophosphatidylcholine and long chain ceramides compared to men. Women presented lower triglycerides in the LS group but slightly higher triglycerides in the IR group. Ceramides with less than 18 carbons were higher in women than men in PGS, ID and IR groups.

Conclusions: Our work demonstrates that not only glucose but also lipidomic profile furthermore differentiated by gender should be included in the analysis of phenotypes for the increased risk of dysglycemia and T2D.

56ASM-0267 | It takes two to tango: The involvement of certain environmental endocrine-disrupting chemicals in male infertility aetiology

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Background: Exposure to pollutants originated from (or used in) industrial processes contributes to the decreased male fertility and reduced reproductive health worldwide. This can be more obvious in populations occupationally exposed and/or living in polluted areas. To this extent, the Portuguese city of Estarreja encloses the 2nd largest chemical complex of the country, and local contamination of heavy metals/metalloids was reported, with a preponderance of arsenic(As) and mercury(Hg).Despite the efforts made to mitigate such contamination, it is still unknown if male fertility is compromised.

Materials and Methods: Both *in vivo* (1) and *in vitro* (2) approaches were used. For (1), 280 samples were collected from men who filled a medical, lifestyle and exposure questionnaire. Several samples were excluded due to eligibility criteria and the remaining were divided in exposed ($n = 10$) and control groups ($n = 88$).For (2),samples were exposed up to 24h (37°C, 5%CO_2, n = 8-10) with As and Hg doses found in seminal fluid of men from Estarreja (ICP-MS) and others described worldwide.

Spermiograms were performed according to the WHO, along with markers for accessory sex glands function. Sperm functional parameters- viability (eosinY),ROS production (DHE), mitochondrial function (JC-1), chromatin/DNA status (Diff-Quik),acrosome integrity (PSA-FITC) and tyrosine phosphorylation (immunofluorescence)-were determined.

Results: (1)No differences between control and exposed groups were detected in semen volume, pH, accessory glands function and sperm concentration, motility and morphology; yet the sample size is small. Similarly, no differences were obtained in As and Hg levels (nM range) in seminal fluid. Nonetheless, and although the values were smaller than reported around the world,(2) showed they were sufficient to synergistically decrease motility, chromatin integrity and produce an increasing trend in ROS production. Higher physiological doses elevated ROS production and decreased mitochondrial function, motility, chromatin and acrosome integrity, tyrosine phosphorylation and viability.

Conclusions: As and Hg levels in Estarreja are similar to the general population. However, certain compounds act synergistically at concentrations found in seminal fluid to compromise male fertility.

This is the first study in Portugal addressing male fertility in an industrial-related scenario.