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S1 – METABOLISM, LIFESTYLES AND LIVER-GUT AXIS

54ASM-0014 | Assessing the utility of metabolite profiles of human serum and urine for determining dietary intake

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Background: Accurately measuring human behaviours impacting health, such as diet, remains challenging even in tightly-controlled studies. Metabolomics has emerged as a potential tool for measuring environmental exposures. This project aimed to discover dietary biomarkers in two human biofluids, serum and urine, using established protocols for ^{1}H-NMR and Mass spectrometry (MS).
Materials and Methods: Fasting serum and urine samples, along with four-day food diaries, were collected from 87 healthy older adults enrolled in the NIDAS dietary validation study. Samples and diaries were collected again 6 months later. Samples were aliquoted, prepared and NMR spectra were obtained using a 600 MHz Bruker ASCEND. Mass spectrometry analysis involved the application of Biocrates p180 kits with a Waters TQ-S instrument. Serum and urine NMR data were processed using Bayesil and ChenomX software, respectively. MS data were processed using MassLynx and MetIDQ. Statistical analyses were conducted in SPSS and R. Food-metabolite correlation coefficients (CC; Spearman) were calculated with the Benjamini-Hochberg multiple comparisons correction procedure applied ($q \leq 0.05$). Potential confounding factors such as age, sex and BMI were examined. Significant associations were further tested by receiver operating characteristic (ROC) analysis, to determine discrimination.
Results: Nine novel serum biomarkers, mostly Phospholipids correlated with dairy, meat and fruit consumption, were discovered and statistically validated. Phosphatidylcholine AA C32:1 correlated closely with dairy ($q = 0.0213$, CC = 0.302). Three novel urinary biomarkers for the consumption of dairy (Alpha-aminoadipic acid – $q = 0.029$, CC = 0.350), coffee (Mandelate – $q = 0.034$, CC = 0.343) and vegetables (Valerylcarnitine – $q = 0.039$, CC = −0.337) intake were discovered. Trigonelline was confirmed as a urine biomarker of coffee consumption ($q = 3.79\text{E-}6$, CC = 0.514).
Conclusions: Specific phosphatidylcholines are strong putative biomarkers for dairy and meat consumption in older adults, and could prove beneficial in epidemiological studies after further validation. Urinary trigonelline appears to be a reproducible marker for coffee intake.

54ASM-0046 | Serum bile acid levels before and after sleeve gastrectomy and their correlation with obesity-related comorbidities

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Background: The rising prevalence of morbid obesity is increasing the demand for bariatric surgery. The benefits observed after bariatric surgery seems to be not fully explained by surgery-induced weight loss or traditional cardiovascular risk factors regression or improvement. Some evidences suggest that bile acid (BA) levels change after bariatric surgery, thus suggesting that BA concentrations could influence some of the metabolic improvement induced by bariatric surgery. In this report, we have characterized circulating BA patterns and compared them to metabolic and vascular parameters before and after sleeve gastrectomy (SG).
Materials and Methods: Seventy-nine subjects (27 males, 52 females, aged 45 ± 12 years, mean BMI 45 ± 7 kg/m^2) SG candidates were included in the study. Before and about 12 months after SG, all subjects underwent a clinical examination, blood tests (including lipid profile, plasma glucose and insulin, both used for calculating HOMA-IR, and glycated hemoglobin), ultrasound visceral fat area estimation,

ultrasound flow-mediated dilation evaluation, and determination of plasma BA concentrations.

Results: Before SG, both primary and secondary BA levels were higher in insulin-resistant obese subjects than in non-insulin resistant obese, and BA were positively associated with the markers of insulin-resistance. After SG, total (conjugated and unconjugated) cholic acids significantly decreased (p 0.007), and total lithocholic acids significantly increased (p 0.017). SG-induced total cholic and chenodeoxycholic acid changes were directly associated with surgery-induced glycemia (p 0.011 and 0.033, respectively) and HOMA-IR (p 0.016 and 0.012, respectively) changes.

Conclusions: Serum BA are associated with glucose metabolism and particularly with markers of insulin-resistance. SG modifies circulating BA pool size and composition. SG-induced BA changes are associated with insulin-resistance amelioration. In conclusion, an interplay between glucose metabolism and circulating BA exists but further studies are needed.

54ASM-0050 | Iron status and arterial stiffness in newly diagnosed hypertensive patients with different degrees of glucose tolerance

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Background: Iron is an important element involved in many biological processes and the ferritin protein has a crucial role for iron storage. Many studies have shown a positive association between ferritin levels and the prevalence of chronic diseases such as essential arterial hypertension (EAH), type 2 diabetes mellitus (T2DM). Arterial stiffness (AS) is directly related with ferritin circulating levels. The aim of the present study was to evaluate if iron status may affect AS in hypertensive patients with different degree of glucose tolerance.

Materials and Methods: We enrolled 462 newly diagnosed untreated hypertensive (HT) patients (mean age 49.6 ± 12.2 years). The study population underwent an oral glucose tolerance test (OGTT); 271 subjects showed a normal glucose tolerance (HT/NGT), 146 presented a glucose intolerance (HT/IGT) and 45 were diabetic (HT/T2DM). Insulin sensitivity was measured by HOMA index. Serum ferritin levels were estimated by immune radiometric assay. AS was evaluated as measurement of carotid-femoral pulse wave velocity (PWV).

Results: Iron serum levels significantly decreased from the first to the third group, on the contrary ferritin and transferrin serum levels significantly increased. PWV and central systolic and diastolic BP values significantly increased from the HT/NGT to the HT/T2DM patients. PWV was significantly correlated with age ($P < 0.0001$), hs-PCR ($P < 0.0001$) and ferritin ($P < 0.0001$), while an inverse correlation was observed with the e-GFR ($P < 0.0001$) and the MATSUDA index ($P < 0.0001$). Ferritin was the first independent predictor of PWV justifying a 23.8% of its variation, also Matsuda index, hs-CRP and e-GFR entered in the model accounting for another 7.7%, 3.9% and 1.4%, respectively. The final model explained a 36.8% of PWV variation.

Conclusions: Our present data demonstrate that ferritin serum levels are strongly associated with AS, in a large cohort of untreated HT patients with different degrees of glucose tolerance.

54ASM-0072 | Efficacy of marine omega-3 phospholipids in alleviating different stages of NAFLD in dietary obese mice

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Background: Omega-3 fatty acids (Omega-3) incorporated in triacylglycerols can alleviate hepatic steatosis, the first stage of non-alcoholic fatty liver disease (NAFLD), but are less effective in non-alcoholic steatohepatitis. Rodent studies suggested that marine phospholipids are superior to Omega-3 as triacylglycerols in terms of liver fat reduction. Here, using the new mouse model of obesity-linked NAFLD, we aimed to determine the effect of marine phospholipids on both hepatic steatosis and non-alcoholic steatohepatitis.

Materials and Methods: Male C57BL6/NCrl mice were kept at 30°C and fed for 24 weeks a lard-based high-fat diet (LHF) or LHF containing marine phospholipids as Krill oil (ω3PL). Moreover, ω3PL or LHF supplemented with Omega-3 triacylglycerols (ω3TG) was also administered to obese mice previously fed with LHF for 8 weeks (therapeutic approach). Both ω3PL and ω3TG were matched for EPA+DHA content (30 mg/g). Mice fed a low-fat chow served as lean controls. Energy balance, plasma markers of liver injury, hepatic triacylglycerol content, gene expression in the liver, and insulin sensitivity (hyperinsulinemic-euglycemic clamp) were analyzed.

Results: LHF increased the triacylglycerol content of the liver 3-fold, while plasma levels of AST and ALT were

elevated 1.6- and 4.2-fold, respectively. Compared to LHF, ω3PL reduced body weight gain by 9%, primarily due to preferential effects on mesenteric adipose tissue. Liver triacylglycerols content was reduced to 54%, as were plasma levels of AST (66%) and ALT(32%). Expression of lipogenic genes was significantly reduced in ω3PL mice, which also showed improved insulin sensitivity. Using a therapeutic approach, the effects of ω3PL on NAFLD phenotypes were similar and on inflammation- and collagen remodelling-related genes even stronger than those seen in the preventive approach. In contrast, no changes were associated with ω3TG.

Conclusions: Our results demonstrate the superior effects of Krill oil on NAFLD-related phenotypes and provide a rationale for using Krill oil phospholipids as a nutritional supplement in NAFLD patients.

54ASM-0073 | Suspected Food intolerances in Functional Gastrointestinal Disorders (FGIDs) and role of probiotics on symptoms: A comparison between an Italian and a Romanian Cohort

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Background: Food intolerances may co-exist and aggravate symptoms in patients with Functional Gastrointestinal Disorders (FGIDs). We profiled lactose and/or fructose intolerances in FGIDs in two similar referral clinics (Clinica Medica "A.Murri", Bari, Italy and Medicala II, Cluj-Napoca, Romania), and studied the effects of two different probiotics mixtures on symptoms in patients with persisting gastrointestinal disturbance despite restrictive diet.

Materials and Methods: After exclusion of major organic diseases (IBD, celiac disease), FGID patients (Rome IV criteria) underwent oral challenge with lactose (25 g) and fructose (35 g) and hydrogen breath test (H2BT, Bedfont, AB Analitica, Padua, Italy). Intolerant patients with persistent symptoms after at least one month in restrictive diet, received probiotic for 30 days (Italian Cohort, ZR: *Bifidobacterium longum* BB536, *Lactobacillus rhamnosus* HN001, Vitamin B6; Romanian Cohort, EQ: *Lactobacillus plantarum* CECT 7484, *Lactobacillus plantarum 7485*, *Pediococcus acidilactici*) in a double blind, placebo-controlled fashion.

Results: 206 patients were screened (Italians 135, mean age 48 ± 3.1 years, 71 Romanians, mean age 52 ± 4 years). In the Italian cohort 75/135 (53%) and 0% of patients were lactose and fructose intolerant, respectively. Symptoms improved in 52/75 (69%) patients on lactose-restrictive diet. The 23 remaining patients, tested with ZR had decreased bloating ($P = 0.028$) and ameliorated constipation ($P = 0.045$) vs. placebo. In the Romanian cohort, 4/71 (3%) and 26/71 (18%) patients were lactose and fructose intolerant, respectively. Symptoms improved in 100% patients on lactose-restrictive and in 8/26 (31%) of patients on fructose-restrictive diet. The 18 remaining patients, treated with EQ, had significantly ($P < 0.0001$) improved constipation, bloating and abdominal pain after 30 days.

Conclusions: Apparently, lactose and fructose intolerances show divergent trends in Italy and Romania. Intolerant patients with persistent symptoms on a restrictive diet, improve during 30 days with probiotics. Further analyses on gut microbiota and dietary profiles are ongoing to better characterize the best therapeutic options.

54ASM-0113 | Role of MAGP-1 in patients with colon cancer in the context of obesity

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Background: Changes in the extracellular matrix (ECM) composition of adipose tissue (AT) in obesity greatly influence the metabolism and growth of tumour cells, providing a favourable microenvironment for tumorigenesis. The ECM protein microfibril-associated glycoprotein 1 (MAGP-1) constitutes a key regulator of AT remodelling during obesity. We aimed to determine its impact on obesity and its associated comorbidity colon cancer (CC).

Materials and Methods: Samples obtained from 77 subjects [22 lean (12 with CC) and 55 with obesity (19 with CC)] were

used in a case-control study. Anthropometric and metabolic variables were analyzed. Gene expression levels of *MFAP2* and *TGFB1* were analysed in visceral AT (VAT). Circulating MAGP-1 and TGF-β levels as well as the concentrations of well-known inflammatory markers were also measured in the different groups.

Results: Significant differences in circulating MAGP-1 concentrations between the experimental groups were observed, being significantly decreased due to obesity ($P < 0.01$) and CC ($P < 0.001$). Consistently, we showed that gene expression levels of *MFAP2* ($P < 0.05$) were downregulated in VAT from obese patients with CC. An opposite trend in *TGFB1* mRNA levels in VAT between the experimental groups was observed. We also found that obesity significantly increased ($P < 0.05$) plasma levels of vascular endothelial growth factor (VEGFA) and WNT1-inducible-signaling pathway protein 1 (WISP1). Osteopontin (OPN) and interleukin (IL)-6 concentrations were increased due to both obesity ($P < 0.01$) and CC ($P < 0.05$). In this sense, obese patients with CC exhibited higher ($P < 0.01$) plasma levels of IL-8 compared with lean patients with CC. Circulating levels of the anti-inflammatory cytokine IL-4 were also affected by obesity ($P < 0.05$) and CC ($P < 0.05$), exhibiting decreased circulating levels in obese and CC patients.

Conclusions: These findings provide evidence about the potential involvement of MAGP-1 in the development of obesity-associated CC probably by its inhibitory effects on TGF-β.

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54ASM-0118 | NLRP3 inflammasome as a mediator of inflammation and extracellular matrix remodelling in obesity-associated non-alcoholic fatty liver disease

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Background: Activation of the inflammasome in adipose tissue and liver is an essential mediator of obesity-induced inflammation, insulin resistance and hepatic diseases. Our aim was to investigate whether the inflammasome in visceral adipose tissue (VAT) and liver is activated in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).

Materials and Methods: VAT and liver samples from 98 subjects for a case-control study were used. Anthropometric and metabolic variables were evaluated. Gene transcripts of components of the inflammasome, inflammation and ECM remodelling were analyzed.

Results: Obese subjects suffering from NASH exhibited higher mRNA levels of *NLRP3* ($P < 0.05$) in VAT compared with volunteers with normal liver and subjects with NAFLD. We found that the presence of NAFLD and NASH was associated with higher expression levels of *NLRP6* ($P < 0.05$) and *ASC* ($P < 0.01$) as well as *IL1B* ($P < 0.01$) in VAT. Although patients with liver disease exhibited increased mRNA levels of *NLRP1* and *IL18*, differences were not statistically significant. No differences were found between patients with or without NAFLD and NASH in the expression of *NLRP1*, *NLRP6*, *ASC* and *IL18* in the liver, but mRNA levels of *NLRP3* ($P < 0.05$) and *IL1B* ($P < 0.01$) were significantly upregulated in the liver of obese patients with NASH. A positive association ($P < 0.01$) between gene expression of *NLRP3* in the liver and the circulating levels of ALT and fibrinogen was found. We also detected that hepatic mRNA expression of *NLRP3* was significantly associated ($P < 0.05$) with the expression of *IL6*, *IL8*, *IL32*, *MMP2*, *MMP9* and *TGFB*.

Conclusions: *NLRP3*, *IL1B* and *IL18* gene expression levels are increased in the liver from obese patients with NAFLD showing that the activation of different components of the inflammasome as well as its main mediator IL-1B in VAT are involved in the development of obesity and obesity-associated NAFLD.

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54ASM-0122 | Is Hyperglycemia the trigger for Diabetes-related Male Infertility?

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Background: Diabetes mellitus (DM) has reached pandemic proportions and is one of the most prominent health issues worldwide. Perilously, this number is steadily increasing among children, adolescents, and young adults. Although DM is known to cause many complications, male fertility is overlooked. Indeed, clinical studies highlight that both hyperglycemia and DM compromise male fertility, although the molecular mechanisms that mediate hyperglycemia-mediated infertility remain to be elucidated. Herein, we studied the impact of increasing glucose concentrations on human sperm physiology and mitochondrial function.

Materials and Methods: Human seminal samples were obtained from healthy men seeking fertility treatment (n = 40). A density gradient centrifugation was performed to isolate spermatozoa. Then, spermatozoa were incubated at 37 °C with increasing glucose concentrations (in mM: 0, 5.5, 11, and 22, which mimics non-physiological, normal, prediabetic and diabetic concentrations) in capacitating conditions for 6 h. Upon incubation, viability and total motility was accessed. Mitochondrial activity was accessed by JC-1 assay. Phosphotyrosine levels were measured to evaluate capacitation. Total ROS production was quantified using the CM-H2DCFDA probe. Oxidative stress (OS) biomarkers (lipid peroxidation, tyrosine nitration and protein carbonylation) were quantified. Culture media was analyzed by 1H-NMR to study metabolic changes induced by increased glucose concentrations.

Results: Our data show that hyperglycemic concentrations promoted not only the maintenance of spermatozoa viability over time, but also increased total motility and promoted capacitation. Notably, we observed an increased mitochondrial activity and an ROS overproduction, although no significant OS-induced damage was observed.

Conclusions: Human spermatozoa are resilient against the direct exposure to hyperglycemic glucose concentrations. Increased mitochondrial activity and total ROS production were not followed by OS-induced damage, suggesting that ROS overproduction may be essential for human spermatozoa capacitation and increased motility. Overall, hyperglycemia induces capacitation and ROS overproduction on human spermatozoa.

54ASM-0123 | The impact of isocaloric meal ingestion containing different fructose/glucose proportions on the sources of plasma glucose production and on the pathways to glycogen synthesis in healthy subjects

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Intermediary Metabolism

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Background: Since the late 60s there has been a sharp increase in the intake of high-fructose corn syrup-55 (HCFS-55) in Western countries. This has been correlated with the increase of insulin resistance, Type 2 Diabetes and fatty liver

disease. We propose to characterize the glucose metabolism in healthy subjects following the ingestion of a test meal which fructose/glucose proportion resembles that of HFCS-55 and compare to a load with low fructose/glucose which has been described to have beneficial effects on glycemic control in diabetic subjects.

Materials and Methods: Twelve healthy subjects were recruited for two studies involving the ingestion of isocaloric test meals with different fructose/glucose proportions. The meals consisted of 50 g 55% fructose/45% glucose or 5% fructose/95% glucose and whey protein dissolved in water. After overnight fasting, subjects were given the test meal having ingested deuterated water (^2H_2O) 2 hr before. Five hundred mg paracetamol were ingested 1 hr before and 1 hr after the meal. Blood glucose was measured before and periodically after the meal. A 30 mL blood sample was collected 3 hr after the meal for analysis by ^2H NMR. Urine was collected at 2-4 hr for paracetamol glucuronide (PG) and body-water (BW) ^2H-enrichment determination. The gluconeogenic contribution to plasma glucose production was estimated from the enrichment of glucose hydrogen 5 relative to BW (H5/BW) and the contributions of direct and indirect pathways to hepatic glycogen synthesis were determined from PG H5/BW.

Results: Glycemic profile was similar for both studies. Gluconeogenic contributions to plasma glucose levels were 65 ± 2 vs. 34 ± 3% for high fructose and low fructose meal content, respectively. The indirect pathway contribution to hepatic glycogen synthesis was similar for both studies.

Conclusions: Our data showed a higher gluconeogenic contribution following ingestion of fructose/glucose proportion matching that of HFCS-55. However, the glycemic control was maintained probably through hepatic autoregulation.

54ASM-0170 | The dangerous "belly" in lean nafld females

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Background: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases. Obesity is a worldwide epidemic which accompanies NAFLD as well as metabolic syndrome and related co-morbidities. However, it has been recently found that not only obese subjects can develop NAFLD and, NAFLD can be found in lean subjects. We explored abdominal adiposity as a predictor of NAFLD in lean subjects.

Materials and Methods: In 39 lean subjects (females: 62%, NAFLD: 23%), liver steatosis (degree: 0-3) and visceral fat (mm) were graded and assessed ultrasonographically. Waist circumference (cm) was measured using a non-stretching tape, following the indications from the Revised National Cholesterol Education Programme-Adult Treatment Panel III (R-ATPIII) and the International Diabetes Federation (IDF). All subjects were stratified according to presence of liver steatosis (NAFLD) and gender.

Results: Liver steatosis was mild in lean NAFLD subjects (1.0 ± 0.0, mean ± SEM). Lean NAFLD were older (51.9 ± 2.9 vs. 34.9 ± 2.9 years, $P = 0.024$) and heavier (24.1 ± 0.3 vs. 21.7 ± 0.4 kg/m^2, $P = 0.043$) than lean healthy subjects. Waist circumference was significantly increased in lean NAFLD when compared to lean healthy females (R-ATPIII: 109.4 ± 12.1 vs. 74.2 ± 1.9 cm, $P = 0.005$; IDF: 93.9 ± 7.0 vs. 69.2 ± 1.3 cm, $P = 0.022$, respectively). Visceral fat was also significantly increased in lean NAFLD when compared to lean healthy females (45.6 ± 6.8 vs. 22.5 ± 2.4 mm, $P < 0.001$, respectively).

Conclusions: In this sub-study, abdominal adiposity (namely, waist circumference and visceral fat) was significantly increased in lean NAFLD females, despite having normal body mass index (< 25 kg/m^2). Although data are still scarce concerning lean NAFLD and their potential related-cardiovascular risk factors, more scientific effort should be put into understanding the complexity of this novel finding in the NAFLD epidemic.

54ASM-0178 | Adipocyte hypertrophy leads to mitochondrial dysfunction in a cell model for adipogenic differentiation

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Background: The changes in lifestyle led to an increasing prevalence of obesity worldwide. Hypertrophy of adipocytes, due to enlargement of cytosolic lipid droplets (LD), represents the main cause of obesity leading to energy metabolism

dysfunction. Here, we investigated *in vitro* the cellular changes associated with adipocyte hypertrophy.

Materials and Methods: The adipocyte hypertrophy model consists of 3T3-L1 pre-adipocytes firstly differentiated into mature adipocytes (48 hours of 1 μM insulin, 1 μM dexamethasone, and 500 μM 3-isobutyl-1-methylxanthine, followed by 7 days of insulin alone), then cultured with long-chain fatty acids (oleate/palmitate) to mimic *in vitro* the obesity condition. For each condition we assessed the intracellular triglyceride (TG) content, the LD size, the ROS production and lipid peroxidation by spectrophotometry and microscopy analyses, and the expression of markers such as PPARg and Adiponectin by real-time PCR. The mitochondrial function was evaluated by using the Oxygraph 2 k (O2K) high-resolution respirometer. The quantification of OXPHOS complexes and of mitochondrial proteins HSP60 and Citrate Synthase (CS) was carried by Western Blot.

Results: Mature adipocytes exposed to oleate/palmitate showed a larger increase in the TG content and LD size, up-regulation of PPARg and adiponectin expression with respect to mature adipocytes, demonstrating hypertrophy. The hypertrophic adipocytes also showed a larger increase in ROS production that reflected on higher MDA levels, a secondary by-product of lipid peroxidation. Moreover, the hypertrophic condition led to a general reduction in mitochondrial oxygen consumption, and a significant reduction in the activities of all the mitochondrial complexes. No significant differences of CS and HSP60 level were observed between mature and hypertrophic adipocytes.

Conclusions: Our findings suggest that adipocyte hypertrophy is associated to changes in a wide spectrum of cell functions. In particular, we observed a reduced mitochondrial activity, but not mitochondrial mass, depending on the hypertrophic condition of adipocytes and elevated oxidative stress.

54ASM-0192 | Adherence to mediterranean diet in southern italy. older do better than younger subjects

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Background: Diet plays a crucial role in the prevention of health. The Mediterranean Diet is scientifically reported as a model of healthy eating, and it is recognized by UNESCO as an intangible cultural heritage of humanity. Several studies demonstrated that the higher adherence to Mediterranean Diet the more beneficial health outcomes, particularly in elderly, with several impacts on cardiovascular diseases, type II diabetes, cancer and overall mortality. Over the last decades, a shift occurred from traditional healthy diets (such as Mediterranean Diet) in younger generations. We thereby aimed to study the link between adherence to Mediterranean Diet and age groups, in relation to body weight and liver steatosis.

Materials and Methods: In 98 subjects (age: 43.6 ± 1.5 years, body mass index: 28.6 ± 0.7 kg/m^2, female: 44%, NAFLD: 63%, obese: 40%), adherence to Mediterranean Diet was assessed by a validated questionnaire (Sofi et al. 2014). Nine typical Mediterranean food products (fruit, vegetables, legumes, cereals, fish, meat and meat products, dairy products, alcohol and olive oil) were scored each on a semi quantitative three-point scale (0-3; max score 18), based on consumption frequency. Subjects were stratified according to age (young < 35 years; adult ≥ 35 years), body mass index (BMI), presence of liver steatosis (NAFLD) and sex. Liver steatosis was assessed and graded by ultrasonography (0-3).

Results: Mean degree of liver steatosis in NAFLD subjects was 1.6 ± 0.1 (mean ± SEM). Adult subjects consumed more Mediterranean food products than young subjects (10.9 ± 0.2 vs. 9.4 ± 0.4, $P = 0.002$). When classifying adult subjects by BMI (normal weight, overweight and obese), normal weight subjects consumed more Mediterranean food products than overweight and obese subjects (12.4 ± 0.5 vs. 10.1 ± 0.4 and 10.7 ± 0.3, $P = 0.002$ and $P = 0.013$, respectively). Furthermore, healthy adults consumed more Mediterranean food products than NAFLD adults (11.9 ± 0.5 vs. 10.6 ± 0.3, $P = 0.030$). There were no significant differences concerning adherence to Mediterranean Diet between genders, and overall the adherence was only sufficient (score: 10.5 ± 0.3).

Conclusions: In our clinical setting in Apulia, adult subjects had more adherence to Mediterranean Diet, as compared to young subjects. In this population, lean and healthy subjects did also consume more Mediterranean food products. Overall, 35% of our cohort was not sufficiently adherent to Mediterranean Diet (score < 10), and almost half of them were young subjects (47%). Local and regional food products should be made available in both public and private institutions in order to promote a healthier consumption of the youth in Southern Italy and overall Mediterranean countries.

54ASM-0197 | Chemical characterization, gastrointestinal motility and sensory evaluation of dark chocolate: a nutraceutical boosting consumers' health

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Background: Chocolate has a pleasant taste and beneficial effects on human health, mediated by lipid and antioxidant components. Effects are specially seen with dark chocolate. We performed a "real-life" comprehensive study, which included chemical analysis of chocolates, sensory evaluation, gastrointestinal symptoms and motility in healthy subjects with two dark chocolates.
Materials and Methods: Healthy volunteers (n = 50) were enrolled. We compared an artisanal chocolate (Choco-A) and an industrial chocolate (Choco-I) for chemical composition (polyphenols and antioxidant activity by Folin-Ciocalteu, DPPH, and ABTS methods assays) and proteomic characterization (by LC-MS/MS analysis, following a bottom-up proteomic approach). Lipidomic and metabolomic profiles were obtained by LC-MS/MS. Sensory evaluation, following chocolate ingestion, was scored on a semiquantitative scale. Gallbladder/gastric motility (ultrasonography), and orocecal transit time (H2-breath test) in response to chocolate were studied in a subgroup of 16 healthy subjects, and compared with an isovolumetric standard liquid test meal (Nutridrink®, Nutricia, Milano, Italy, 300 kcal, 200 mL).
Results: Chocolate is rich in polyphenols, amino acids derivatives and fatty acids (stearic and myristic acids, showing the highest abundance). Main proteins were the 21 kDa seed protein and Vicilin A protein. For agreeability, Choco-A scored higher than Choco-I for smell, texture, and taste. Both Choco-A and Choco-I induced similar gastrointestinal motility. However, gallbladder emptying was less, residual gastric antral area was smaller, and orocecal transit time was shorter in response to chocolates than test meal.
Conclusions: From a chemical point of view, dark chocolate is a valuable source of polyphenols and amino acid derivatives. Saturated fatty acids, such as stearic and myristic acids, prevailed on the others detected and among proteins the 21 kDa seed protein along with Vicilin A protein were those mainly identified after MS/MS analysis. Chocolate, in the artisanal form, has better organoleptic characteristics that suggest a favourable consumption compared to the industrial chocolate. The ingestion of a small quantity of chocolate induces a mild gallbladder, gastric contraction and fast transit time revealed by a valid fermentation curve.

54ASM-0226 | Heterogeneity in HCC: role in prognosis and insights into guidelines

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Background: Heterogeneity of cancer is an emerging trend in the diagnostic and prognostic evaluation of tumors. Our aim was to gain new insight into elements of heterogeneity of hepatocellular carcinoma (HCC) and their correlation to response to therapy and survival.
Materials and Methods: Retrospective evaluation of 160 patients affected by HCC enrolled in Clinica Medica "A. Murri" (Bari, Italy) for which a 12-month follow-up was available.
For each patient, we attended age at the diagnosis of HCC and at the time of the conclusion of the follow-up, gender, presence of comorbidities, etiology and stage of the underlying liver disease, the patient's clinical condition (Performance Status), staging according to the criteria of the BCLC system, and finally the date of the last follow-up or of any death.
For the purpose of this work, on the basis of the numer of nodules of tumor, patients were subdivided into: UNIFOCAL, MULTIFOCAL and MULTIFOCAL POST (unifocal at the diagnosis and multifocal during the follow-up).
Results: 73% of males and 96% of females are >60 years; unifocal tumors were more frequent among survived patients and multifocal among dead subjects; diabetes was more common in dead patients; obesity and overweight was present in more than 50% of subjects; elevated values of AFP predicted a short survival as well as total volume of tumor; unifocal were present in younger age and elevated serum glucose was typical of multifocal and multifocal-post subjects.
Conclusions: HCC is a disease of old age; in women, it appears at an older age than in men; diabetes and obesity can be considered two unfavorable prognostic factors; unifocal neoplasms have a better prognosis than multifocal ones; AFP values at diagnosis are predictive of a less favorable prognosis; the BCLC classification system is unable to fully describe the heterogeneity of the hepatocellular carcinoma.

54ASM-0227 | Look at the patient and not only to guidelines: the case of HCC

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Background: Lenvatinib is an orally available, small-molecule multikinase inhibitor of VEGF receptors 1-3, FGF receptors 1-4, PDGF receptors α, RET, and KIT, that showed activity in hepatocellular carcinoma (HCC). In a randomized phase 3 trial, it was demonstrated that lenvatinib was non-inferior to sorafenib in overall survival in first-line advanced HCC. The most common adverse events were hypertension (68.6%), diarrhea (62.8%), anorexia (51.5%) and weight reduction (49.1%). In the phase 3 select study, hypertension was reported in 72.8% of treated patients, the median time to onset in treated patients was 16 days.

Materials and Methods: We selected three patients affected by HCC, classified on the basis of Child Class and staged by BCLC criteria; all of them underwent to previous ablative treatment.

To our evaluation all patients presented advanced stage (BCLC B-C), no longer candidates for loco-regional therapies, Child-Pugh class A and performance status 0; all aged > 65 years.

Among the comorbidities, all patients had hypertension. In one patient with no pressure control, anti-hypertensive therapy was increased, obtaining normal values before starting lenvatinib.

Following guidelines, they were submitted to oral Lenvatinib therapy (12 mg /day for bodyweight ≥60 Kg or 8 mg /day for bodyweight ≤60 kg) as first-line strategy, with the recommendation to monitor blood pressure.

Results: After about 20 days from the start of therapy, all patients presented hypertensive crises up to 200/95 mmHg. One of them was hospitalized, for an N-STEMI with hypertensive crisis. Lenvatinib was immediately suspended.

Conclusions: Clinical trial results are not always reflected in clinical practice. In the case of lenvatinib, the simple monitoring of the PA is not sufficient at all. It is necessary to activate a much more stringent relationship between physician and patient and select candidate patients, possibly excluding those subjects with poor control of pressure.

54ASM-0244 | The leaky gut assessed by the pan-enteric four-sugar oral test solution. The impact in frequent metabolic disorders and diseases in clinical practice

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Background: A well-functioning intestinal permeability (IP) is essential for maintaining health. Acute and chronic local and systemic diseases can cause inflammation, intestinal dysbiosis, and metabolic abnormalities. We studied IP in the entire gastrointestinal tract in several diseases by a novel four-sugar oral test solution.

Materials and Methods: 206 subjects (122 female) were enrolled (120 lean, overweight and obese subjects without additional diseases, LOO; 33 irritable bowel syndrome, IBS; 25 functional constipation, FC; 5 Familial Mediterranean Fever, FMF; 12 naïve celiac disease, CD; 11 with immunological, hematological or other tumors, IHT). Subjects drank a 200 mL water solution containing sucrose 20 g (stomach-duodenum study), lactulose 5 g + mannitol 1 g (small intestine study), sucralose 1 g (colon study). Urinary recovery of selectively absorbed sugar probes during 6-hr was measured in urinary samples by triple quadrupole mass spectrometry and HPLC (Mass-Q Gastropack, AB Analitica, Padua, Italy). Cut-off normal values were <0.15%, <1.5% urinary recovery and <0.03 ratio for stomach, colon and small intestine, respectively.

Results: In LOO subjects, colonic permeability increased significantly with BMI, waist, neck, hip circumferences and liver steatosis. Small intestinal and colonic permeability were abnormally increased, respectively, in 21% and 15% (IBS), in 12% and 16% (FC), and in 60% and 20% (FMF). Small intestinal permeability was abnormally increased in 42% of CD patients, including one patient with increased gastric, small intestinal and colonic permeability. Small intestinal permeability was also abnormally increased in 30% of IHT patients.

Conclusions: The study of intestinal permeability provides clues for a better knowledge of intestinal barrier integrity in health and disease. The four-sugar oral test solution indicates that initial metabolic disorders, as well as frequent diseases observed in clinical practice, are associated with a leaky gut at different levels (mainly small intestine and colon). Such abnormalities might contribute to impair pathways involving nutrients, microbiota, mucosal integrity, and the ongoing inflammatory response. Further studies need to assess intestinal permeability systematically in larger groups, to address the role of therapeutic lifestyle interventions and restore intestinal permeability.

54ASM-0250 | Efficacy of the probiotic B. breve BR03 L. Rhamnosus LR04 (Prolactis) on symptoms and dysbiosis index in patients with irritable bowel syndrome – A pilot study

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Background: Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits. The pathophysiology of IBS is multifactorial and includes defects of gastrointestinal motility, visceral hypersensitivity, mucosal immune system activation, postinfectious causes, genetic background, and psychological factors. In addition, gut dysbiosis and bacterial overgrowth might affect food sensitivity and symptoms perception. We recently reported beneficial effects of combination of *B. longum* BB536 *L. rhamnosus* HN001 plus vit. B6 on IBS symptoms. We aim to evaluate the short-term efficacy of a novel probiotic on IBS symptoms and dysbiosis.
Materials and Methods: This was an open label study in IBS patients (Rome IV criteria) undergoing a trial with *B. breve* BR03 and *L. Rhamnosus* LR04 plus vit. B1, B2, B3, B5, B6, B9, B12 for 10-day (Prolactis START, Omegapharma, IT) followed by *B. breve* BR03 *L. Rhamnosus* LR04 plus fruit-oligosaccharide (Prolactis LT, Omegapharma, IT) for 14 days. At baseline and 24-hr after end of therapy, symptoms (bloating, abdominal pain on a visual analogue scale (0-100 mm), stool consistency (Bristol stool scale score 1-7), and dysbiosis index on fecal samples (GA-map™ Dysbiosis test, Biohit Healthcare Srl, Milano, Italy) were measured.
Results: At baseline, abdominal pain and bloating averaged (SEM) 72.8 ± 4.5 and 77.1 ± 3.7 mm, respectively. Bristol score was 3.1 ± 0.5. Patients had variable microbiota profile differing from reference population for Actinobacteria, Bacteroides, Firmicutes, Proteobacteria, Tenericutes, Verrucomicrobia phyla. After treatment, abdominal pain and bloating decreased significantly to 45 ± 8.7 mm ($P = 0.03$) and to 58.6 ± 4.3 mm ($P = 0.005$), respectively, while microbiota profile were closer to reference population for Actinobacteria (*Bifidobacterium spp.*), Bacteroides, Firmicutes (*Bacilli* spp., *Ruminococcus gnavus, Faecalibacterium prausnitzii, Lactobacillus spp.*), and Proteobacteria (*Shigella spp.; Escherichia spp.*).
Conclusions: IBS is a complex disease with a heterogeneity of symptoms and microbiota profile. A short-term trial with a novel combination of *B. breve* BR03 *L. Rhamnosus* LR04 improved symptoms and could reshape gut microbiota. This pilot but promising study deserves larger controlled trials, looking at differences according to gender, body mass index, age, diet, and duration of treatment.

54ASM-0268 | Estimating pentose phosphate pathway activity from the analysis of hepatic glycogen 13C-isotopomers from [U-13C]fructose

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Background: In the fed state, sugar phosphate flux through the pentose phosphate pathway (PPP) is a key requirement for *de novo* lipogenesis since it generates NADPH equivalents for fatty acid synthesis and/or elongation. Recently, a method for quantifying PPP activity in circulating glucose or liver glycogen by analysis of hexose ^{13}C-isotopomers generated from [U-^{13}C]glycerol was developed[1]. Since both fructose and glycerol are metabolized to glucose or glycogen via common triose phosphate intermediates, we reasoned that [U-^{13}C]fructose could also be used to measure PPP activity. Among other things, this allows PPP activity to be measured under conditions of high fructose feeding, where it contributes acetyl-CoA to the synthesis of fatty acids via *de novo* lipogenesis. To date, it is not known to what extent fructose also contributes to PPP activity.
Materials and Methods: Six male C57/BL6 mice fed with standard chow were provided with a 55/45 mixture of fructose and glucose. This sugar mixture was presented at 30% w/v in the drinking water for 12 weeks. On the final evening, the fructose component was enriched with 20% [U-^{13}C]fructose. The mice were allowed to feed naturally overnight and then sacrificed. Livers were freeze-clamped and glycogen was extracted and derivatized for ^{13}C NMR spectroscopy. From the ^{13}C NMR analysis of glycogen ^{13}C isotopomers, PPP activity was estimated using the same analysis previously developed for [U-^{13}C]glycerol[1].
Results: Hepatic glycogen was enriched with ^{13}C-isotopomers from the metabolism of [U-^{13}C]fructose to glycogen via triose and hexose phosphates. The distribution of ^{13}C-isotopomers in carbons 123 of glycogen was significantly different from those in carbons 456, indicating that the PPP was active under our experimental conditions. Applying the analysis of Jin et al.[1] to our ^{13}C-isotopomer data, we estimated that 9 ± 1% of the fructose that had been metabolized to glucose-6-phosphate was utilized by the PPP.
Conclusions: Fructose is considered to be a highly lipogenic sugar and it has been previously shown to contribute substantially to *de novo* lipogenesis in mouse models. In this study,

we demonstrate that it also supports PPP activity, thereby contributing NADPH for the reductive formation of fatty acids during *de novo* lipogenesis.

54ASM-0276 | Efficacy and safety of a synergistic molecular complex of polysaccharides and minerals (Neo-Bianacid®) on gastroesophageal reflux. A pilot study.

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Background: Gastroesophageal reflux disease (GERD) has high prevalence, different clinical presentations leading to morbidity, and economic consequences. GERD can have a high impact on quality of life (QoL). In naïve patients with mild and intermittent symptoms, and no evidence of erosive esophagitis, the step-up approach includes weight control, lifestyles and dietary modification, antacids and/or sodium alginate, low-dose histamine 2 receptor antagonists (H2RAs), and proton pump inhibitors (PPI). By contrast, the step-down approach is reserved to patients with severe GERD, esophagitis, and includes PPI at high dose, possibly followed by progressive decrease of therapy to the best, clinically and histologically effective therapy. NeoBianacid® (NB) is a novel medical device containing Poliprotect®, a synergistic molecular complex of polysaccharides and minerals with muco-adhesive properties and therefore acting as a surface agent. Definitive studies are still lacking in this respect in patients with initial and mild GERD symptoms. We aim to evaluate the efficacy and safety of NB on symptoms associated with initial and mild GERD symptoms, and in patients with severe symptoms already on PPI therapy.
Materials and Methods: An open label study short-term study was designed in three groups of patients observed for 30 days. Group 1(n = 30): mild GERD symptoms on NB alone; Group 2 (n = 4): severe GERD symptoms on PPI alone; Group 3 (n = 6): severe GERD symptoms on PPI plus NB. At baseline and follow-up, the visual analogue scale (VAS, 0-100 mm) was used to record symptom scores for heartburn, reflux, epigastric pain, dyspepsia, and general impact on QoL. The number of weekly reflux episodes was recorded. NB was given 1 pill to be dissolved orally after each meal plus one at bedtime (i.e. 4 times/day). PPI therapy could include omeprazole, lansoprazole, and esomeprazole pantoprazole at 20, 30, 20, and 40 mg/day, respectively.
Results: In Group 1 (NB alone), baseline symptoms, QoL were mild, with a small weekly reflux episodes. In 70% of patients all parameters improved contributing to a significant difference with baseline. In Groups 2 and 3 the baseline scores were higher than Group 1 and decreased significantly after therapy. Absolute and percent change of number of weekly reflux episodes were greater with PPI+NB (Group 3), than PPI alone (Group 2). NB was well-tolerated.
Conclusions: The present study supports the efficacy and safety of Neo-Bianacid in patients with mild GERD, in those initially refusing therapy with PPI, or in association with PPI.

54ASM-0292 | Lactose intolerance in Southern Italy. Profiling population with clinical suspicion by gender, age and symptoms

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Background: Lactose intolerance (LI) is highly prevalent in Southern Italy, but information on general characteristics of patients and prevalence of gastrointestinal (GI) symptoms in subjects with suspect LI are lacking, so far.
Materials and Methods: During 24 months, 264 subjects (mean age 34 ± 1 years; BMI 24.04 ± 0.29 kg/m^2; 179F, 85M) without GI comorbidities (inflammatory bowel diseases, celiac disease, diverticulosis, gastro-esophageal reflux disease) underwent H_2-Breath Test (H_2BT) with 25 g lactose. According to referral, patients were grouped for: A) lower GI symptoms (abdominal pain, bloating, diarrhea, constipation, altered bowel habit); B) upper GI symptoms (heartburn, epigastric pain, nausea/vomit); C) both lower and upper GI symptoms; and D) no GI symptoms.
Results: LI was diagnosed in 126 (47.7%) subjects (grade I, II, III: N = 40 (31.8%), N = 58 (46.0%), N = 28 (22.2%), respectively) and was more frequent in females (75%). Malabsorbers were 45 (17.1%), while 93 (35.2%) subjects had a normal H_2BT. Gender, age and BMI were comparable across all groups. Group A, B, C and D were 73.0%, 3.2%, 17.5% and 6.3% in LI and similar to normal H_2BT. Group A, however, was more represented in malabsorbers than LI and normal H_2BT ($P < 0.05$).
Among symptoms in group A, abdominal pain was more frequent in LI than normal H_2BT (60.3% versus 44.1%, $P = 0.017$) and the prevalence increased with grades of LI (grade I: 52.5%; grade II: 62.0%; grade III: 67.9%; $P < 0.05$ versus normal H_2BT). The prevalence of bloating was similar between LI and normal H_2BT (52.4% versus 54.8%, respectively), but increased significantly with LI grade II (56.9%) and III (60.7%), compared with normal H_2BT ($P < 0.05$). The prevalence of reported diarrhea was similar between LI (48.4%), malabsorbers (51.1%) and normal H_2BT (52.7%).
Conclusions: In our setting, LI is more frequent in females. Lower GI symptoms represent the most common clinical

presentation for referral of all categories. Abdominal pain and bloating (not diarrhea) are distinctive features in patients with established LI, but not in malabsorbers, as compared with normal H_2BT.

54ASM-0361 | NAFLD and diet in a romanian center from Transylvania

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Background: NAFLD (Nonalcoholic fatty liver disease) is characterized by increased fat accumulation in the hepatocytes of the liver parenchyma and it is one of the most frequent etiology of the chronic liver disease. NAFLD is linked to obesity, type 2 diabetes mellitus, and factors of the metabolic syndrome, like insulin resistance. NAFLD can progress to non-alcoholic steatohepatitis (NASH), and further on to liver cirrhosis and hepatic carcinoma. Diet has an important key role in the development of NAFLD.
Materials and Methods: On the period of 5 years, between 2015-2019, there were diagnosed 1456 patients with NAFLD (mean age 57.4 years, 64.3 males) and 139 with NASH (mean age 65.1, 72.3% males). In the Gastroenterology Clinic. Among these patients questionnaires were given to 100 patients to evaluate the life style and diet. All the patients had given the consent to participate in the study, and had to answer to 36 questions.
Results: From the patients 42% had a BMI between 25 and 29.5%, 16% were obese and 16% had a BMI between 18.6-24.9. 38% of the patients were sedentariness, 32% had moderate physical activity, 28% regular physical activity and only 2% had increased activity (fitness).
35% of the patients were skipping breakfast and 51% have the most important meal of the day in the evening, including soup and also second course.
At the questions about diet, 26% of the patients preferred fried meat, 32% grill meat and 42% boiled or steamed.
At the question about the kind of the meat 42% of the patients preferred the pork meat, 26% poultry meat, 16% fish and 16% beef meat. Most of the patients declared that they have 3 times/week vegetables and also fruits; only 10% are eating everyday.
76% of the patients declared that they are not drinking any more alcohol after the diagnosis, but the rest are still consuming. Most of the subjects were not smoking, but drinking coffee between 1 and 2 cups/day.
Conclusions: NAFLD is increasing in our country, most important factors seems to be the typical diet rich in pork meat, mostly fried and the lack of physical activity. 64% of the patients intend to change the life style, much more interested being the women, but 36% declared that diet is not important. Nutritionists have to increase the awareness about the importance of diet and life style.

54ASM-0365 | Eradication rates in Helicobacter pylori infected subjects. Time to abandon empiric treatments in Southern Europe

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Background: H.pylori eradication is strongly affected by various factors, including the ongoing antibiotic resistance. We describe a 'real life' scenario inpatients managed for H. pylori-related conditions, living in a southern Italian region (Apulia), an area with clarithromycin resistance >15%.
Materials and Methods: 3,183 subjects were studied in two tertiary referral centers in Apulia. Analyses included: reason for referral, H. pylori infection rates (13C-urea breath test (UBT) or upper endoscopy), and eradication rates following distinct regimens previously prescribed or prospectively prescribed (such as the bismuth-based quadruple therapy Pylera®, recently marketed in Italy).
Results: Over 80% of patients were referred by general practitioners (60% naïve subjects). The overall infection rate was 39.1% and differs in asymptomatic patients (20.6%) and in H. pylori-related symptoms/clinical conditions (40.3%). In the 1161H.pylori+ve patients receiving therapy, the overall eradication rate (ER) was 73.2%(ITT). Observed ER varied greatly across different regimens: 60.3% (2nd line levofloxacin), 59.6% (unconventional), 70.6% (7-day triple), 72.6% (7-day undefined), 86% (10-day sequential) and 96.9% (ITT, 10 day Pylera®, 1st to 5th line regimens given to 275 patients).
Conclusions: An heterogeneous 'real life' scenario in Southern Europe shows that H.pylori+ve patients are put at risk of poor outcomes and points to the need of a susceptibility-based therapy according to guidelines and local microbial resistance. In the present setting (i.e. high clarithromycin resistance), despite the high observed eradication rate, sequential therapy should not be recommended (absent in guidelines, unneeded antibiotic). Bismuth-based quadruple treatment (1st, 2nd or subsequent lines) yield the highest eradication rates.

S2 – IX MITOESCI: MITOCHONDRIAL BIOLOGY IN HEALTH AND DISEASE

54ASM-0109 | Lactate attenuates synaptic transmission and affects brain rhythms featuring high energy expenditure

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Background: Lactate is a three-carbon, electron-rich metabolite that can be produced and released by various cell types of the body. In utilizing cells, lactate is linked to oxidative ATP synthesis in mitochondria, which requires conversion back to pyruvate through the redox enzyme lactate dehydrogenase, the tricarboxylic acid cycle and molecular oxygen serving as the final electron acceptor at the respiratory chain. Neurons are generally capable of lactate uptake and utilization in mitochondria.
Materials and Methods: We used tissue oxygen concentration measurements, optogenetic stimulation, field potentials and whole-cell patch-clamp recordings.
Results: A major issue is that lactate metabolism has been rarely linked to physiological neuronal activity that underlies information processing in the cortex. Lactate utilization in excitatory neurons and inhibitory interneurons, which can – based on their functions – substantially differ in electrophysiological and bioenergetic properties, has been barely explored. Related to that, the necessity of fast glycolytic ATP supply during neuronal signaling is not well established. We show that lactate disturbs electrical gamma and theta-gamma oscillations in hippocampal networks by either attenuation or recurrent neural bursts. Bursting is suppressed by elevating the glucose fraction in substrate supply. Lactate does not affect electrical sharp-wave-ripple activity featuring lower energy use. Lactate increases the oxygen consumption during the network states, reflecting enhanced oxidative ATP synthesis in mitochondria. Finally, lactate attenuates dendritic excitation in fast-spiking, GABAergic interneurons as well as perisomatic inhibition in glutamatergic pyramidal cells, whereas axonal action potential generation is regular.
Conclusions: In conclusion, lactate is less effective and potentially harmful during gamma-band rhythms, likely because of some obligatory ATP delivery through fast glycolysis required for proper synapse strength.
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54ASM-0120 | Activation of SIRT1 enhances metabolic and bioenergetic performance of mouse Sertoli cells

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Background: Sirtuins have been identified as active participants in the metabolic coordination of various cellular systems. Among all deacetylases, sirtuin 1 (Sirt1) is the most extensively studied. It has been reported that Sirt1 deficiency compromises spermatogenesis, which is highly dependent on the metabolic cooperation established between Sertoli cells and germ cells. Still, little is known about its role in the nutritional support of spermatogenesis, particularly Sertoli cells metabolism. Thus, we hypothesized that SIRT1 is a key player in Sertoli cells metabolism, which may impact spermatogenesis.
Materials and Methods: TM4 Sertoli cells (mSCs) were exposed to increasing concentrations of a SIRT1 activator (YK-3-237). The metabolic profile of mSCs was determined by Nuclear Magnetic Resonance spectroscopy and quantification of lipid accumulation by oil red staining. Mitochondrial function was assessed by evaluating mitochondrial complexes expression and mitochondrial potential (JC-1 assay). The oxidative damages were also analyzed (lipid peroxidation) by the slot-blot technique.
Results: Exposure to the SIRT1 activator (1 nM to 10 μM) presented no cytotoxicity to mSCs. Metabolically, when SIRT1 was activated (10 μM) there was an increase in glucose and pyruvate consumption, suggesting an enhancement of the glycolytic flux, which was translated into an increase in lactate production. Additionally, there was a decrease in lipids accumulation, suggesting their metabolization in mitochondria. In fact, exposure to SIRT1 activator (10 μM) favored mitochondrial function, prompting mitochondrial

membrane hyperpolarization. Under these conditions, mSCs exhibited higher levels of lipid peroxidation.

Conclusions: Modulation of SIRT1 leads to alterations in the metabolic and bioenergetic profiles of mSCs. Notably, activation of SIRT1 enhances the metabolic performance of mSCs, stimulating both the glycolytic and the oxidative metabolism. Hence, SIRT1 is an essential control point for the nutritional support of spermatogenesis by SCs, a key feature in spermatogenesis.

54ASM-0143 | Purified F-ATP synthase forms a Ca2+-dependent high-conductance channel matching the mitochondrial permeability transition pore

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Background: The molecular identity of the mitochondrial megachannel (MMC)/permeability transition pore (PTP), a key effector of cell death, remains controversial. In 2013 Giorgio et al. provided evidence that dimers of mammalian F_OF_1 ATP synthase purified from native gels can form the PTP/MMC but the exact molecular mechanism is still unclear.

Materials and Methods: F-ATP synthase was purified from bovine heart mitochondria employing the mild, high-affinity detergent LMNG. This preparation was characterized by means of clear native PAGE, SDS-PAGE, mass spectrometry, and negative stain electron microscopy. The presence of channel activity was assessed after incorporation of the protein into planar lipid bilayers (PLB), by means of direct reconstitution or proteoliposomes fusion, in the presence of Ca^{2+} and specific PTP activators.

Results: By combining highly purified, fully active bovine F-ATP synthase with preformed liposomes we show that Ca^{2+} dissipates the H^+ gradient generated by ATP hydrolysis. After reconstitution of the same preparation into planar lipid bilayers we observed Ca^{2+}-induced currents matching those of the MMC/PTP. Currents were stabilized by benzodiazepine 423, a ligand of the OSCP subunit of F-ATP synthase that activates the MMC/PTP, and were inhibited by typical PTP inhibitors, such as Mg^{2+} and adenine nucleotides. Channel activity was insensitive to inhibitors of the adenine nucleotide translocase (ANT) and of the voltage-dependent anion channel (VDAC).

Conclusions: These findings resolve the long-standing mystery of the MMC/PTP and demonstrate that Ca^{2+} can transform the energy-conserving F-ATP synthase into an energy-dissipating device.

54ASM-0173 | Differentiated SH-SY5Y cells as a cellular model to study mitochondrial physiology and dynamics

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Background: Primary neuronal cultures or IPS cells-derived neurons represent important models to study mitochondrial metabolic alterations that lead to neurodegeneration and neuronal cell death. However, these models have disadvantages and ethical concerns. An easy, fast and efficient cell model to study mitochondria-related neurodegeneration is thus needed. We characterized the phenotype of neuroblastoma cell line SH-SY5Y after differentiation into a neuronal-like phenotype, focusing on the evaluation of mitochondrial physiology.

Materials and Methods: Undifferentiated SH-SY5Y cells were cultured +/- 10 μM retinoic acid (RA) for 3 days. The efficiency of differentiation was evaluated by the percentage of NeuN-positive nuclei by immunofluorescence. Phenotypical characterization was assessed by the levels of glutamatergic, cholinergic and GABAergic markers by qRT-PCR. Cell morphological features were assayed by the levels of MAP2, Enolase2, Nestin and βIII-Tubulin by qRT-PCR and immunofluorescence. Mitochondrial network was stained using TOM20 and mitochondrial area, content and network extension was evaluated. Finally, mitochondrial oxygen consumption rate (OCR) was assessed in differentiated cells acutely treated with Rotenone, using the Seahorse XFe96 Analyzer.

Results: Differentiated cells showed an increase in mature neuronal markers NeuN, MAP2 and Enolase2 compared to undifferentiated cells. Additionally, βIII-Tubulin, an immature neuronal marker, increased, while Nestin levels showed no alterations. From the transcriptome profile, differentiated cells did not appear to belong to a specific neuronal family. Whilst glutamatergic and cholinergic markers seemed to be upregulated, GABAergic markers did not reveal a uniform tendency. Regarding mitochondrial network extension and content, differentiated cells displayed an increase

in mitochondrial content and maximal area per cell. Lastly, acute rotenone (0.25 μM) treatment led to a decrease of OCR parameters except proton leak and non-mitochondrial respiration.

Conclusions: Although 3 day-differentiated cells did not exhibit a fully mature/differentiated neuronal phenotype, constituting a heterogeneous cellular population, their morphological features are useful for studying neurotoxicity processes, mitochondrial dynamics and bioenergetic impairment, representing an *in vitro* alternative to study mitochondrial dysfunction-related pathologies.

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54ASM-0195 | P19 embryonal carcinoma stem cells exhibit a metabolic remodeling upon ANT2 silencing

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Background: The inner mitochondrial membrane adenine nucleotide translocase 2 (ANT2) is responsible for the ATP uptake into the mitochondria, and is known to be upregulated in several cancers, being associated with cell proliferation, impaired apoptosis, and metabolic changes. Thus, we aimed to determine if ANT2 expression is increased in P19 embryonal carcinoma stem cells (P19SCs) and whether its differential expression levels can lead cancer stem cells to remodel their metabolic profile.

Materials and Methods: P19SCs were differentiated using retinoic acid (RA, 1 μM) producing a mixture of cells with mesoderm and endoderm properties (P19dCs). Differentiation and pluripotency markers, and ANT2 levels were evaluated in P19SCs and their differentiated counterparts (P19dCs) by Western Blot. P19SCs transfected with control or ANT2-siRNA were evaluated for different parameters regarding cell viability, mitochondria remodeling and cellular metabolism. Sulforhodamine B, Western blot, confocal microscopy and seahorse XF^e96 cell Mito Stress assays were used. Statistical comparisons were evaluated using Student's t-test. Differences with $P < 0.05$ were considered statistically significant.

Results: Upon RA treatment, OCT4 levels decreased by 94%, and TROMA-I and β-3-Tubulin levels increased by 96% and 410% ($P < 0.01$), respectively, in P19dCs. ANT2 was highly expressed in P19SCs, with P19dCs having less 73% of expression ($P < 0.05$). In P19SCs-ANT2-silenced cells, ANT2 expression was diminished by 90% ($P < 0.001$), accompanied by decreased cell viability, especially at 72 hours post-transfection ($P < 0.05$). Furthermore, the protein levels of hexokinase II and pyruvate dehydrogenase kinase diminished by 32% and 45% ($P < 0.05$), respectively, as well as the mitochondrial membrane potential ($P < 0.05$). Basal and maximal respiration, proton leak and ATP production-linked respiration decreased by 23-30% in ANT2 silenced cells ($P < 0.001$). Moreover, mitochondrial transcription factor A expression levels were slightly decreased (28%), although no differences in TOM20 were found, the latter suggesting similar mitochondrial mass.

Conclusions: Our findings demonstrate that ANT2 expression is increased in P19SCs comparatively to P19SCs. Additionally, P19SCs ANT2-silenced cells present a decreased cell proliferation versus control cells, which is accompanied by a reduction in oxygen consumption and mitochondrial membrane potential. These data suggest that silencing ANT2 promotes a remodeling towards a less oxidative metabolism, with some evidence pointing to reduced glycolytic flux, although having the same amount of mitochondria, which may sustain the lower proliferation rate. Therefore, ANT2 silencing could be a promising target towards CSCs niche.

54ASM-0204 | FAD synthase deficiency: a severe mitochondrial myopathy searching for novel therapeutic strategies

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Background: Multiple Acyl CoA deficiency (MADD, OMIM #231680) is a heterogeneous disorder affecting multiple mitochondrial flavoproteins, which involves energy metabolism, and particularly β-oxidation of fatty acids. Presentations range from severe forms with multiple organ failure, to later-onset lipid storage myopathy, with excellent response to riboflavin (vitamin B2), the precursor of the redox flavin cofactors, FMN and FAD. Genetic testing

for MADD comprises analysis of *ETFA*, *ETFB*, *ETFDH* and genes involved in riboflavin transport and flavoprotein biosynthesis. Deficiency of FAD synthase caused by *FLAD1* variations was recently identified as a cause of MADD (now lipid storage myophathy, LSMFLAD, OMIM #255100) with potential effect of riboflavin treatment, especially in patients with milder *FLAD1* variations (Olsen RKJ, et al. AJHG 2016; 98:1130-1145).

Materials and Methods: Human fibroblasts were cultured as in (Ryder B, et al. JIMD Rep. 2019;45:37-44). Flavin homeostasis was studied by HPLC. Protein and transcript levels were measured by Western Blotting and PCR. Bioenergetics, ROS measurements and worm *flad1* silencing were performed, as in (Liuzzi VC, et al. BBA. 2012;1820:521-31).

Results: The derangements of flavin homeostasis were studied in fibroblasts of patients expressing *FLAD1* pathological variants. An impairment of mitochondrial bioenergetics, mainly due to reduction in the level of succinate dehydrogenase flavoprotein subunit, and an increase in cellular ROS and peroxiredoxin 3 levels were observed, suggesting an active response to stress conditions. Interestingly, these changes were accompanied by a secondary transcriptional regulation of the riboflavin transporter (RFVT2).

A similar behavior was found in a *C. elegans* model mimicking FAD synthase deficiency, obtained by silencing the worm orthologue of *FLAD1*.

Conclusions: FAD synthase deficiency is a pathological condition which severely alters mitochondrial flavoproteome and bioenergetics in both human cells and nematodes. Secondary derangements of RFVT2 might explain the therapeutic response to riboflavin treatment. The silenced worm is a promising model for testing alternative therapeutic strategies for this mitochondrial myopathy.

54ASM-0205 | High fat vs High sugar: the role of mitochondria on time-dependent hepatocellular cytotoxic effects

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Background: Non-alcoholic steatohepatitis (NASH), one of the deleterious stages of non-alcoholic fatty liver disease, remains a major cause of liver-related morbidity and mortality worldwide. Overload of high fat-high sugar diets demands a great metabolic effort from the liver, which can lead to unregulated processes of energy homeostasis. The deleterious sequence of cellular events in the liver includes fatty acid and/or carbohydrates overload followed by an increased ROS production, mitochondrial damage and ER-stress, resulting in activation of either survival or pro-apoptotic pathways. In the current work, we investigate time-dependent cellular effects of different FA overload strategies in the presence or absence of carbohydrates (fructose) in order to produce a feasible *in vitro* model for study mitochondrial impairment during NAFLD/NASH.

Materials and Methods: Time-dependent effects of supra-physiological concentrations of palmitic acid (PA) or a mix of free fatty acids (FFA) in the presence or absence of fructose (F) were studied on human hepatoma-derived cell line HepG2 by measuring intracellular neutral lipid content and ROS levels, mitochondrial O_2 consumption and morphology, and caspases activity and cell death.

Results: PA and FFA-treatments induced a time-dependent increase in neutral lipid content, which was paralleled by an increase in ROS levels. Fructose by itself neither increased intracellular lipid content nor aggravated the effects of PA or FFA. Increased lipid accumulation and intracellular ROS levels leads to mitochondrial dysfunction. Mitochondrial dysfunction comprises not only altered mitochondrial membrane potential and morphology but also a decrease in oxygen consumption rates, specially with PA. Consequently, supra-physiological PA alone or in combination with F prompted the activation of caspase-dependent pathways leading to a time-dependent decrease in cell viability.

Conclusions: A more comprehensive understanding of chronological mitochondrial dysfunction in different NASH/NAFLD in vitro cell models allowed to attain a feasible model in which mitochondria dysfunction increased in the order: CTL <F < FFA ≤ FFA+F < PA ≤ PA + F.

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54ASM-0236 | Relating oligomerization of in-membrane proteins to their function

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Background: Oligomerization of proteins is often related to their function. However, many completely opposite cases have been reported in literature where formation of protein clusters is rather indicative of their biological dysfunction. As an example may serve a fibroblast growth factor 2 (FGF2) or well-known proapoptotic protein Bax, that oligomerize on the plasma/mitochondrial membrane into functional membrane pores. Researchers have used all kinds of biophysical approaches to characterize such in-membrane aggregates and intuitively connected the obtained oligomerization numbers with the formation of a functional pore. The question, however, is, whether this intuitive connection indeed holds.
Materials and Methods: GUVs with a plasma membrane like lipid composition were generated based on electroswelling using platinum electrodes (Garcıa-Saez et al., 2009). The determination of oligomeric states was based on a comparison of the brightness of a cluster to that one of a monomer. GUVs were selected and classified with regard to the presence (small tracer AlexaFlour532 equilibrated between lumen and exterior) or absence (no tracer in the vesicle interior) of membrane pores.
Results: To study the process of protein oligomerization in detail, we developed a new functional assay based on fluorescence correlation spectroscopy (FCS) which can be used to determine the size of in-membrane protein oligomers by measuring the brightness of individually diffusing oligomers, and correlated the size of these oligomers to membrane permeability. Moreover, we monitored the protein oligomeric state on the same lipid vesicle over time, which allowed for correlating the formation of pores to protein oligomerization. We show that solely determining oligomerization numbers of proteins gives very limited information on the formation of pores. On the contrary, the statistics of our data implies that some published in-membrane aggregation numbers might not be physiologically relevant.
Conclusions: The statistics of our data implies that some published in-membrane aggregation numbers might not be physiologically relevant.

54ASM-0241 | Effects of calorie restriction on mitochondrial biogenesis, repair and dynamics in aged rat liver

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Background: Aging involves the progressive functional decline of tissues, which includes the tissue-specific dysfunction of mitochondria, resulting in a reduced ATP synthesis. A marked age-related decrease in mitochondrial function is reported in rat liver. Calorie restriction (CR) is, so far, the only intervention able to delay or prevent the onset of several age-related changes.
Materials and Methods: Livers from 18-month-old, 28-month-old and 32-month-old *ad libitum*-fed as well as 28-month-old and 32-month-old calorie-restricted rats were used to analyze mitochondrial DNA (mtDNA) content and damage by different techniques based on Real Time PCR (rt-PCR) and end-point PCR. The activity of citrate synthase (CS) by an enzyme spectrophotometric assay and the expression of proteins involved in repair and dynamics by western blots are determined.
Results: A positive effect of CR on the age-related loss of mtDNA content has been found, while the eventual influence on mtDNA damage is currently under study. The counteracting action of CR on the described age-related decline of CS activity has been shown. Also the expression of proteins active in the base excision repair (BER) of mtDNA and in the fission/fusion balance of mitochondrial dynamics has been demonstrated to be reported by CR to values comparable to those of younger animals.
Conclusions: The preliminary results from ongoing determinations in rat liver strongly support a role of CR in counteracting the age-related decline in mitochondrial biogenesis, repair and dynamics and help to clarify some of the mechanisms induced by this anti-aging strategy.

54ASM-0243 | Mitochondria-targeted triphenylphosphonium conjugates of acetylsalicylic acid derivatives as potential antibacterial agents

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Background: Recent research data indicate that compounds containing the triphenylphosphonium group may be an alternative in the treatment of infections caused by antibiotic-resistant bacteria. The action of these compounds is directed to the bioenergetic processes in the cell, which are associated with the cell or mitochondrion membranes.

Materials and Methods: Acetylsalicylic acid (AcSA) and its derivatives (acetylsalicylic triphenylphosphonium(TPP) conjugates: phosphonioalkyl(C3-6, C9 and C10) – compounds 1-6, respectively) were studied. S.aureus ATCC 29213 and E.coli ATCC 25922 were used. Bacteriostatic activity was studied by the microdilution method in Mueller-Hinton broth. Sterile serially diluted compounds (250-0.12 μM) were mixed with the bacterial inoculum in 96-well plates and incubated for 24 hours at 37 °C under moderate shaking. Bacterial growth was analyzed visually in three parallel wells by appearance of turbidity/precipitate and, in addition, by the change in color of phenol red indicator (18 μg/mL).

Results: The compounds were found to effectively prevent bacteria growth at micromolar concentrations only in the case of S.aureus but not E.coli. Clear increase in the bacteriostatic activity from ca. 31 to 1 μM (1-6) was observed upon increase in the linker length of compounds. Both unmodified AcSA and its bromoalkyl derivative similarly were almost inactive (MIC = 125–500 μM) indicating that the bacteriostatic effect of the conjugates is determined by alkyl-TPP group.

Conclusions: Given the MIC value (24 h) and higher IC50 value for human skin fibroblasts (4-5 μM, 72 h), the compounds 5,6 can be potentially used to treat staphylococcus infection. Their MBC value was 2 μM, suggesting bactericidal mechanism of their ability to kill bacteria.

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54ASM-0266 | Overreduced intracellular state detected in Cu-ATSM brain imaging and fibroblasts from prodromal Huntington disease carriers

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Background: Huntington's disease (HD) is a neurodegenerative autosomal dominant disorder caused by an expansion of CAG repeats in the *HTT* gene. Neurodegeneration occurs mainly in medium spiny neurons in the striatum, but other brain regions have been linked to cardinal symptoms, including the globus pallidus, thalamus, amygdala, cerebellum and subthalamic nucleus (STN). The PET imaging agent diacetylbis(4-methylthiosemicarbazonate) copper^{II} [Cu^{II}(ATSM)] (^{64}Cu-ATSM) is useful in identifying elevated reductive state induced by mitochondrial impairment. This study aims to correlate cerebral redox changes using ^{64}Cu-ATSM PET with mitochondrial and metabolic features analysed in fibroblasts from premanifest/early and stage 3 HD patients and controls.

Materials and Methods: Fibroblast-derived from skin biopsies of patients were obtained to further analyse mitochondrial function and metabolism, levels of reactive oxygen species and mitochondrial biogenesis.

Results: Premanifest/early HD carriers exhibited increased mitochondrial basal and maximal respiration, accompanied by enhanced proton leak. In contrast, mitochondrial respiration in fibroblasts from late stage HD patients, in general, persisted unaltered demonstrating a general decrease in mitochondrial respiration. Notably, cellular and mitochondrial ROS production increased with HD progression. Furthermore, mtDNA copies were significantly lower in late stage vs presymptomatic patients. PET images demonstrated higher SUV in premanifest/early HD carriers in putamen, STN and cerebellum, whilst in the caudate SUV decreased with disease severity. Brain regional ^{64}Cu-ATSM standardized uptake values were compared between groups and correlated with clinical and mitochondrial characteristics Interestingly, ^{64}Cu-ATSM uptake in cerebellum, basal ganglia and putamen in HD patients was directly correlated with fibroblast's mtDNA

copy number. Additionally, increased SUV in total brain was correlated with higher basal and maximal respiration.

Conclusions: Based on these data, premanifest/early HD carriers have higher mitochondria number with abnormal function, responsible for enhanced ROS production. PET analysis of ^{64}Cu-ATSM radiotracer shows large potential in detecting overreduced intracellular state in prodromal stages, linked to defective mitochondrial function in HD.

54ASM-0271 | Estrogens inhibit RANKL-induced early stimulation of complex I and mitochondria function in osteoclast progenitors

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Background: Loss of estrogens at menopause increases the risk of osteoporosis and consequently the risk for bone fractures. Estrogens maintain bone homeostasis by decreasing osteoclast number. However, the molecular mechanisms of the anti-osteoclastogenic effects of estrogens remain unclear. Estrogens can increase or decrease mitochondria activity in different cell types, while RANKL - the essential factor for osteoclast differentiation - increases mitochondrial mass during osteoclastogenesis. Here, we examined whether 17β-estradiol (E2) alters mitochondrial activity during osteoclasts differentiation.

Materials and Methods: Bone marrow-derived macrophages were cultured with M-CSF and RANKL (30 ng/mL) in presence and absence of E2 (10^{-8}M). Osteoclast number was assessed by the tartrate-resistant acid phosphatase (TRAP) assay. Cellular metabolic profiling was evaluated by measuring oxygen consumption and extracellular acidification rates using the Seahorse XFe96 Extracellular Flux Analyzer. The ENLITEN ATP bioluminescence detection kit was used to measure ATP levels. A gradient BN-PAGE was used for in-gel Complex I activity staining.

Results: Our results showed that the presence of E2 for the first 24 h, of the 5 day cultures needed to develop osteoclasts, was sufficient to decrease osteoclast number. An increase in mitochondrial complex I activity in osteoclast precursors was observed as early as 6 hours following exposure to RANKL. Mitochondria respiration and ATP production were also stimulated by RANKL at 24 h, well before an effect on mitochondria content could be detected. Addition of E2 to the cultures abrogated the stimulatory actions of RANKL on complex I activity and mitochondria function.

Conclusions: Our results suggest that estrogens inhibit osteoclast formation by preventing the stimulatory action of RANKL on complex I activity.

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54ASM-0277 | Mitochondrial compensatory response in coeliac patients and in Caco-2 cells treated with pepsin-trypsin-digested gliadin

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Background: Coeliac disease (CD) is a gluten-sensitive autoimmune disorder. Gluten toxicity encompasses a wide spectrum of target organ functions, including activation of the immune response and triggering of oxidative stress. This study investigated the inflammatory status and the redox balance in patients with active CD, and evaluated whether alteration of mitochondrial function is involved in the disease. The interplay between mitochondria and gluten-induced oxidative stress has been further examined in intestinal epithelial Caco-2 cells exposed to pepsin-trypsin-digested gliadin.
Materials and Methods: Blood samples from sixteen CD patients and sixteen healthy controls were investigated for IL-1b and IL-8 plasma concentrations, for total and MnSOD enzyme activities, induced TBARs levels, and for lymphocyte mtDNA content. In the kinetic in vitro study Caco-2 cells were exposed for 24 hours to pepsin-trypsin-digested gliadin, alone or in combination with the antioxidant 2,6-di-tbutyl-p-cresol (BHT), and the effects on mitochondrial biogenesis and mtDNA content and integrity were studied. Cells ability to recover from stress was determined after 24 hours and 48 hours of incubation in the culture medium.
Results: Patients showed cytokines concentrations higher than controls. Patients also had a higher content of induced TBARS, along with higher total SOD, and MnSOD. Lymphocyte mtDNA content in patients was twofold higher than in controls, supporting the induction of mitochondrial biogenesis. In Caco-2 cells, gliadin triggered a rapid increase of PGC-1α and Peroxiredoxin III proteins, and mtDNA amount. As for the effects of gliadin on mtDNA integrity, D-loop appeared to be more fragile than Ori-L and ND1/ND2 regions. BHT was able to counteract the effects of gliadin.
Conclusions: Our results highlighted the mitochondrial involvement in CD and the effects of gliadin-induced oxidative stress on mitochondria, providing valuable evidence that might improve the knowledge of the pathophysiology of gluten-related disorders.

54ASM-0290 | Activation of mTOR pathway during short-term preservation increases spermatozoa glycolytic activity

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Background: Infertility is becoming a major health problem, affecting up to 15% of reproductive-aged couples worldwide and the search for assisted reproductive techniques (ART) has been increasing. Furthermore, male-only factor is responsible for 20-30% of infertility cases. The success of ART is dependent upon sperm quality, which decreases after sperm collection. Mammalian target of rapamycin (mTOR) plays an important role in the regulation of several cellular processes, including cellular metabolism. We hypothesized that by activating the mTOR pathway, the metabolic activity of spermatozoa would be altered, particularly concerning glycolysis, resulting in an improvement in sperm quality during short-term preservation.
Materials and Methods: For this study, spermatozoa were obtained from 17 non-smokers patients with normal sperm parameters. Spermatozoa were placed in sperm preservation media (SPM) or SPM supplemented with an activator or an inhibitor of mTOR at 37°C for 30 and 180 minutes. Then, sperm quality was assessed using WHO guidelines and the media collected and analyzed by ^{1}H Nuclear Magnetic Resonance.
Results: Through spectra analysis, we quantified the consumption and production of metabolites to assess if activation or inhibition of mTOR signaling pathway alters spermatozoa metabolism during short-term storage. The results show that incubation with mTOR activator increases lactate production by spermatozoa by 14% and 13%, at 30 and 180 min, respectively, whereas incubation with the inhibitor decreased this production by 16% at 30 min, and 6% at 180 min, when compared with spermatozoa preserved in normal SPM. Additionally, a significant improvement in sperm quality,

particularly in sperm motility, was observed with the addition of the mTOR activator.

Conclusions: Our data suggest that activation of mTOR pathway during short-term preservation at 37°C of spermatozoa stimulates glycolytic metabolism, while its inhibition diminishes it. Moreover, the mTOR enhancer resulted in an improvement in sperm quality. These results suggest an important role of mTOR in sperm quality, possibly mediated by its effect in cellular metabolism.

54ASM-0304 | Co-administration of liraglutide and ghrelin ameliorates brain mitochondrial energy metabolism in the R6/2 mouse model for Huntington's disease

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Background: Huntington′s disease (HD) is an incurable, autosomal dominant neurodegenerative disease, characterized by motor/cognitive deficits, mitochondrial dysfunction, diabetes and cachexia. We hypothesized that co-administration of liraglutide (an effective anti-type 2 diabetes (T2D) and anorectic drug) with the orexigenic hormone ghrelin alleviates brain mitochondrial dysmetabolism and cognitive deficits in HD. Hence, we aimed to evaluate the effect of peripheral treatment with liraglutide and ghrelin on brain cortical mitochondrial (energy) metabolism and motor function in the early symptomatic HD R6/2 mice.

Materials and Methods: We used early symptomatic HD R6/2 mice to evaluate the effect of a 2-week subcutaneous co-injection of liraglutide and ghrelin on the brain cortical lactate levels, mitochondrial respiratory chain complexes' activities (by colorimetry) and adenine nucleotide levels (by HPLC), alongside their motor function (through the paw clasping and open-field tests).

Results: Liraglutide *per se* or plus ghrelin attenuated peripheral T2D characteristics in R6/2 mice. Liraglutide plus ghrelin stimulated their brain cortical mitochondrial respiratory chain complex IV, and reduced lactate and AMP levels (the later may contribute to the improvement in R6/2 mouse brain energy status). Though, neither the paw clasping score nor the locomotor/exploratory activities were significantly recovered by the treatment.

Conclusions: Liraglutide plus ghrelin may improve brain mitochondrial metabolism, eventually slowing HD progression. Nevertheless, this issue deserves further elucidation.

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54ASM-0326 | ALDH2/4-HNE balance during cold storage of fatty liver grafts is modulated by polyethylene GLYCOL 35

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Background: The cumulative injury resulting from ischemia and cold preservation must be minimized to achieve full recovery of liver function after transplantation, especially in steatotic grafts. Institut Georges Lopez (IGL-1) solution, which contains polyethylene glycol 35 (PEG35) as an oncotic agent, has been considered a good alternative to University of Wisconsin (UW), the gold standard in liver transplantation. The aim of this study is to focus on the role of PEG35 in the IGL-1 solution.

Materials and Methods: In order to evaluate the protective role exerted by PEG35 on steatotic liver graft preservation, two different solutions were applied, IGL-1 and IGL-0 (the same composition as IGL-1 but without the presence of PEG35). Fatty livers from male obese Zücker rats were flushed with either IGL-1 or IGL-0 solution. Cold static preservation (4°C) was performed in the respective solution for 24 h. Serum transaminases levels (AST/ALT) were measured. Liver mitochondrial ALDH2 activity and content were evaluated. Moreover, the levels of toxic aldehyde adduct (4-HNE), lipoperoxidation and oxidized proteins were also assessed by western blot. Endoplasmic reticulum stress and ischemic injury (measured by HMGB1) was also evaluated.

Results: The presence of PEG35 in the original IGL-1 solution reduced the AST/ALT levels when compared with IGL-0 solution (without PEG35). The higher ALDH2 activity, observed in IGL-1 preserved livers, was responsible for the prevention of toxic aldehydes (4-HNE) and lipoperoxidation. Furthermore, the presence of PEG35 in the IGL-1 solution was well correlated with increased nitrites/nitrates (as a consequence of e-NOS activation) and changes in HMGB1 and GRP78 protein expression.

Conclusions: These data confirm that PEG35 plays a key role in increasing liver mitochondrial ALDH2 during cold graft preservation, leading to the subsequent positive ALDH2/4HNE balance responsible for toxic aldehyde/lipoperoxidation generation. PEG35 also promoted vasodilator factors (nitric oxide), which may improve the well-known exacerbation of microcirculation in steatotic grafts. To sum up, PEG35 seems to be a therapeutic agent suitable for limiting cold ischemic injury, especially in fatty liver grafts.

54ASM-0328 | Maternal obesity-induced oxidative damage impairs macroautophagy and unbalances mitochondrial dynamics

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Background: The liver is a metabolically active organ with multiple functions in energetic and nutritional body homeostasis during pregnancy. Hepatic mitochondria adaptations are essential to the metabolic health and pregnancy fate. Nutritional stresses can alter mitochondrial function, leading to hepatic dysfunction. Maternal obesity (MO) prevalence is increasing worldwide. Hepatic alterations during pregnancy due to MO can affect the maternal hepatic metabolism and might lead to the development of immediate and/or long-term pathologies. The objective of this study was to investigate the maternal hepatic mitochondria remodeling due to MO during pregnancy.

Materials and Methods: We used a well-established and characterized MO ovine model consisting in a 50% increase of the recommended global nutrient intake from 60 days before conception, throughout gestation. At 90% of the gestation time, ewes were euthanized under general anesthesia and maternal hepatic tissue collected. Mitochondrial protein expression was assessed by Western blot, enzymatic activities through spectrophotometric assays, and NAD^+/NADH ratio using a luminescent assay. Data were evaluated using unpaired t-tests between MO ($n = 8$) and control ($n = 10$) groups, with $P \leq 0.05$ considered statistically significant.

Results: MO increased lipid peroxidation 81% (MDA levels; $P = 0.0015$) and the NAD^+/NADH ratio 35.9% ($P = 0.006$). These alterations were concomitant with an increase in macroautophagy measured by the LC3-II/LC3-I ratio (+380%; $P < 0.0001$). No alterations were observed in the mitochondrial mass (mtDNA copy number, citrate synthase activity and TOM20 protein expression). MO increased the expression of proteins involved in mitochondrial fission, reduced the expression of proteins involved in mitochondrial fusion (+34.3% of Fis1, $P = 0.049$, and −51% in OPA-1 protein expression, $P = 0.001$), and decreased PKA activity (40%; $P = 0.0007$).

Conclusions: MO during pregnancy leads to hepatic mitochondrial redox state alterations and induces mitochondria degradation and an unbalance in mitochondrial dynamics. These alterations can exacerbate mitochondrial dysfunction and contribute to aberrant cellular homeostasis, possibly increasing the risk of future hepatic diseases. Monitoring liver function during the challenging period of pregnancy can provide new insights to understand later-developing hepatic diseases.

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54ASM-0338 | Maternal obesity during pregnancy: Are there implications for maternal hepatic mitochondrial activity?

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Background: Changes in maternal liver function in normal and abnormal pregnancy are poorly studied. Approximately 3% of pregnancies are complicated by some form of liver disease, with possible negative outcomes for both mother and child. Obesity-induced, non-alcoholic fatty liver disease (NAFLD), is one of the most common hepatic diseases in developed countries. Obesity and NAFLD incidence rates are rising worldwide in reproductive age women. We hypothesized that maternal obesity (MO) during pregnancy could stress hepatic metabolism. Since mitochondrial bioenergetics play a crucial role in hepatic cellular function, our goal was to characterize the effects of MO during gestation on hepatic mitochondrial function.
Materials and Methods: To determine the impact of MO on hepatic mitochondria during pregnancy, mitochondrial respiratory chain (RC) complex activity was quantified in liver extracts from a Rambouillet-Columbia ovine model of obesity during pregnancy (MO; n = 8). Obesity was induced by feeding 150% of feed eaten by controls (C; n = 10) from 60 days previous to conception until caesarean section under general anesthesia to obtain maternal liver at 90% of gestation. Mitochondrial mass was determined by spectrophotometric citrate synthase activity, and protein expression for mitochondrial respiratory chain (RC) complexes by Western blot. RC activities were measured spectrophotometrically. C and MO were compared by Mann-Whitney and non-paired student t-tests; *P*-value $\leq$ 0.05 was considered statistically significant.
Results: MO increased maternal liver weight by 17.0% at necropsy. Protein expression for MO mitochondrial respiratory chain complex II subunits SDHA and SDHB decreased 60.1% and 31.0%, respectively, and complex II activity decreased 24.3%. MO complex I and IV activity increased 52.9% and 105.0%, respectively; expression of complex IV subunit mtCO1 decreased 29.0%. Citrate synthase activity, an indirect measurement of mitochondrial mass, was similar between groups.
Conclusions: Maternal hepatic metabolic demands differ in MO and C, resulting in alterations in mitochondrial respiratory chain activity. We conclude that altered mitochondrial function increases predisposition to hepatic disease in MO gestation.
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54ASM-0340 | Culture conditions affect mitochondrial morphology and cellular metabolism of cancer cells

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Background: Studies of cultured cancer cell lines can yield insights into cancer cell metabolism and biology while facilitating the discovery of new drugs. However, candidate drugs experience a high failure rate in pre-clinical rodent models, and non-physiological cell culture conditions contribute to this problem. Recently, two research groups developed growth media (Plasmax)[1] based on the human serum metabolome that much more faithfully reproduces the environment cancer cells experience *in vivo.* In addition to medium composition, standard cell culture does not take into account the potential roles of oxygen in cancer cell biology; while O_2 levels in culture typically 18-19%, they are 1-5% in most tissues in vivo. O_2, in part via the production of reactive oxygen, affects cell biology. To improve our understanding of how standard (DMEM and 18% O_2) versus physiological (Plasmax and 5% O_2) cell culture conditions affect cancer cells, we characterized mitochondrial network characteristics, reactive oxygen species production, and cellular metabolism in several well-studied cancer cell lines (PC3, MCF7, Huh7, SaOS2) growing in DMEM or Plasmax at either 5% or 18% O2.
Materials and Methods: We measured cellular H_2O_2 efflux using an Amplex Red/horseradish peroxidase assay. Also, mitochondrial morphology and abundance were quantitatively measured using MiNA, the Mitochondrial Network Analysis tool recently developed in our laboratory following staining mitochondria with Tetramethylrhodamine, methyl ester (TMRM). Finally, the Seahorse XFe96 Analyzer (Agilent Technologies, Mississauga, ON) was used to measure cellular and mitochondrial oxygen consumption rates (OCR) under our experimental condition.
Results: We found that culture conditions had significant effects on all of these parameters. For example, mitochondrial abundance was significantly higher in Huh7 and SaOS2 cells when grown at 18% O_2 in plasmax. Interestingly, basic and

maximal oxygen consumption rate were different depending on the level of O_2 and media.

Conclusions: Non-physiologic cell culture conditions can introduce artifacts and reduce our ability to extrapolate to the Invivo conditions.

54ASM-0348 | Impaired mitochondrial carrier activities lead to myopathies

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Background: Recessive mutation in nuclear gene SLC25A1 coding for mitochondrial citrate carrier (CIC) have been identified in two patients affected by myasthenia and impaired neuromuscular junctions (NMJ). Furthermore, lack of mitochondrial glutamate transport by homozygous mutation of aspartate/glutamate carrier (AGC1) was associated to inflammatory myopathy in Dutch Shepherd dogs.

Materials and Methods: 1) To investigate the role of CIC in NMJ function we used worm c. elegans as animal model. Using the CRISPR/CAS9 approach we obtained stable lines of *Caenorhabditis elegans* knocked-out in the SLC25A1 ortholog. The effects of inactivation of SLC25A1 gene on NMJ was investigated studying worm locomotion by the motility assays and measuring the levels of acetylcholine (Ach) in whole worms.

2) The role of mitochondrial AGC1 in dog myopathy was investigated by i) functional transport studies of mutated and wild-type proteins, ii) metabolomics analysis of muscles of affected dogs.

Results: 1) The SLC25A1 knock-out worms exposed to levamisole, a nicotinic acetylcholine receptor agonist, that causes continued stimulation of the worm muscles, showed resistance, indicating a decreased level of acetylcholine in the synaptic cleft of knock-out than wild-type worms. The analysis of Ach in whole knock-out worms showed that neurotransmitter was less than in the wild-type c. elegans. The qPCR analysis showed presynaptic genes involved in acetylcholine metabolism in NMJ of *C. elegans* are induced in knock-out strains. The levamisole resistance was, at least in part, rescued by the expression of wild-type human SLC25A1 under the control of a neuron-specific promoter.

2) A pathological variant of AGC1 gene was detected by whole exome sequencing in affected dog and resulting in leucine to proline substitution at amino acid 349. The functional reconstituted recombinant mutant AGC1 into proteoliposomes demonstrated a markedly decreased transport activity compared to wild-type. Furthermore, metabolic analysis on dog muscle biopsies showed an elevated oxidized glutathione and decreased intramitochondrial NADH/NAD+ ratio.

Conclusions: Our data highlighted the role of mitochondrial carriers in muscular dysfunctions. In this study we demonstrate that 1) SLC25A1 deficiency affects acetylcholine synthesis and neuromuscular transmission. Our data validated the worm *C. elegans* as a suitable animal model for further molecular and cellular investigations of the NMJ transmission. 2) the decreased AGC1 transport activity affects the transfer of cytosolic reducing equivalent to mitochondria, inducing a strongly oxidizing intramitochondrial redox potential and leading to the proinflammatory muscle milieu.

54ASM-0357 | Triphenylphosphonium-linoleic acid to promote tetralineoylcardiolipin formation in a Tafazzin knockout mouse myoblast model of Barth Syndrome

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Background: Barth syndrome (BTHS) is a rare, X-linked disease caused by mutations in the TAZ gene. The TAZ gene encodes an acyltransferase, tafazzin, which is the primary enzyme involved in cardiolipin (CL) remodeling. CL is an essential mitochondrial lipid that is highly enriched in the inner mitochondrial membrane where it plays a role in mitochondrial dynamics and oxidative phosphorylation. CL acyl chain composition is highly specific with linoleic acid (LA, 18:2n6) constituting 80-90% of total CL acyl chains in mammalian cardiac mitochondria. Tetralinoleoyl-cardiolipin (TLCL) is the most abundant CL species, and is required for mitochondrial function. Loss of tafazzin function results in reduced TLCL content and mitochondrial dysfunction. Monolyso-CL-acyltransferase-1 (MLCLAT-1) may account for up to 20% of CL remodelling to TLCL, but fails to compensate in BTHS. Triphenylphosphonium (TPP^+) is a lipophilic cation that has become widely used to target bioactive molecules to the mitochondrial matrix and associated membranes. TPP-conjugated molecules accumulate in the matrix according to the inner membrane potential. In this study, we synthesized TPP-LA to target LA to mitochondria. TPP-LA was synthesized with a linker arm that is subject to esterase cleavage to release free LA in mitochondria. We hypothesize that this will increase the mitochondrial matrix pool of LA, promoting the production of TLCL via MLCLAT-1.

Materials and Methods: We employed a TAZ knockout C2C12 mouse myoblast model of BTHS and isogenic wild-type cells to evaluate the effects of TPP-LA administration on mitochondrial and cellular function. We used our recently developed Mitochondrial Network Analysis (MiNA) tool to evaluate mitochondrial network characteristics due to tafazzin knockout and TPP-LA. Myoblast differentiation was evaluated by myosin heavy chain expression after 7 days in growth factor depleted medium.

Results: Our preliminary data demonstrate that tafazzin knockout increases mitochondrial fragmentation that may be recovered by TPP-LA administration. Additionally, tafazzin knockout does inhibit differentiation and TPP-LA administration fails to rescue this, despite increasing myoblast differentiation in wild-type C2C12 cells.

Conclusions: TPP-LA provides a prototype for treating mitochondrial diseases caused by aberrant phospholipid metabolism. Whereas a single bolus dose of TPP-LA was used in the experiments described here, repeated doses over an extended time period may be required. In actively dividing C2C12 cells, synthesis of new mitochondria occurs at a relatively fast rate. Therefore, it may be informative to study TPP-LA effects over longer periods with differentiating or differentiated cells. We are now investigating the effects of concentration and duration of treatment with TPP-LA on mitochondrial network structure and bioenergetics.

54ASM-0374 | Biological activity of brassica by-products in cell models of lipid toxicity

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Background: A large amount of waste is generated within the different steps of the food value chain, representing a big loss of both natural resources and plant material. Many parts of edible plants are in fact, not sold for consumption and ends up as massive waste. A good example is *Brassica* by-products. A growing concern in the Western world, obesity results from incorrect lifestyles and comprises a large array of co-morbidities, including fatty liver disease. Active search for possible natural-based therapies for obesity complications is underway. The objective of this work was to investigate the effect of three different extracts from *Brassica oleracea* var *italica* by-products (leafs, stalk and inflorescence) in models of oxidative stress and lipotoxicity on human HepG2 cells.

Materials and Methods: Human hepatoma HepG2 cells were used as cellular model for hepatocytes, the protective effects of leaves, stalk and inflorescence extracts from different parts of *Brassica oleracea*, at 2 different concentrations (1 and 10 μg/mL) were tested against 100 μM of pro-oxidant tert-butyl hydroperoxide (tBHP) and free fatty acid (FFA, 0.25 and 1 mM)-induced cytotoxicity. Cell metabolic viability and cell mass were measured by using the resazurin reduction and sulforhodamine B assays, respectively. Lipid accumulation was measured using Nile Red staining assay and normalized using the sulforhodamine B assay.

Results: Preliminary data show that pre-incubation with 10 μg/mL stalk extract (E2.10) for 48 hours had a protective effect on tBHP-induced cytotoxicity, preventing HepG2 metabolic viability loss by ~16% when compared with cells treated with tBHP alone. Also, pre-incubation for 48 hours with 1 μg/mL (E2.1) and 10 μg/mL of stalk extract and 1 μg/mL of inflorescence extract (E3.10) prevented cell mass loss in HepG2 by about 14.1%, 17.6% and 17.4%, respectively, when compared with cells treated with tBHP alone. On the other hand, co-incubation with E2.10 and E3.10, at a concentration of 0.25 mM for 24 hours, reduced FFA accumulation in HepG2 cells.

Conclusions: Further studies are underway to understand not only the mechanism(s) of action, but also the main phytochemicals responsible for the observed effect.

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54ASM-0379 | Beneficial effect of mitochondriotropic antioxidants on oocyte maturation and embryo production

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Background: Gametes and embryos are particularly susceptible to reactive oxygen species (ROS) and their regulation is

mandatory to the success of assisted reproductive technologies (ART). Novel antioxidant molecules directed to mitochondria may reduce excessive ROS production avoiding their deleterious action. In this study, we investigated, for the first time, the effect of two new mitochondriotropic antioxidant molecules, $AntiOxBEN_2$ and $AntiOxCIN_4$, in the prevention of oxidative stress of bovine oocytes and embryos.

Materials and Methods: These new molecules were supplemented to the *in vitro* maturation medium of oocytes (n = 1651 and n = 1062) at doses of 10, 20, 50 and 100 μM during 22 hours in two experiments (6 sessions each). A control group without supplementation was performed simultaneously. Mature oocytes were subjected either to *in vitro* fertilization with bovine capacitated sperm or to evaluation of nuclear maturation (aceto-lacmoid, nuclear dye) and mitochondrial polarization (JC1, fluorescent probe). Cleavage and embryo developmental rates and D7/D8 embryo quality were determined. Data were analyzed using the PROC GLIMMIX and PROC MIXED.

Results: An improvement of nuclear maturation progression was achieved with both molecules in a dose dependent manner ($P \leq 0.05$). All concentrations of $AntiOxCIN_4$ enhanced the mitochondrial polarization of mature oocytes. $AntiOxBEN_2$ supplementation increased the cleavage rate in the concentration of 10 μM when compared to the other groups ($P \leq 0.003$) and doubled blastocyst production rate.

Conclusions: The novel mitochondria-directed antioxidants, $AntiOxBEN_2$ and $AntiOxCIN_4$, improved different aspects of oocyte maturation and embryo production, implying different mechanisms of action. Although the results are still preliminary, they suggest that the inclusion of mitochondriotropic antioxidants in the maturation medium should be a strategy to be implemented in ART.

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54ASM-0396 | Morphological and functional mitochondrial remodeling in human skin fibroblasts cultured with different metabolic fuels

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Background: In vitro identification of mitochondrial stressors is important for the assessment of chemical safety and is influenced by cell culture conditions. Traditional cell culture protocols favour glycolytic ATP production and decrease cellular dependence on mitochondria. In order to detect mitochondrial defects associated with toxicity or disease conditions, it is necessary to increase the cellular reliance on mitochondria-generated ATP. Our objective was to characterize mitochondrial remodeling in normal human dermal fibroblasts (NHDF) in different conditions of metabolic fuel availability.

Materials and Methods: NHDF were cultured in DMEM containing 25 mM Glucose (HGm) and gradually adapted to either DMEM containing 5 mM Glucose (LGm) or to glucose-free galactose-containing DMEM (OXPHOSm). Oxygen consumption rates (OCR) and extracellular acidification rate (ECAR) were measured using a Seahorse XFe96 Analyzer. Phenotypic metabolic microarrays were performed using an Omnilog (Biolog), while mitochondrial network morphology was quantified in confocal microphotographs of cells labelled with MitoTracker Red, using the Mitochondrial Network Analysis (MiNA) tool for the Fiji distribution of ImageJ. Toxicity of mitochondrial inhibitors was assessed using CellTiter-Glo assay from Promega. Statistical significance was accepted for $P < 0.05$, using Kruskal-Wallis with Dunn's post test.

Results: NHDF cultured in HGm and LGm showed low sensitivity to mitochondrial inhibitors. Adaptation to OXPHOSm resulted in increased sensitivity to mitochondrial inhibitors, which was associated with increased mitochondrial mass, suggested by a 23% increase in mitochondrial footprint and 27% increase in summed branch length, as compared to HGm. Mitochondrial activity was also increased, as suggested by the increase in OCR parameters (Basal Respiration (38%), ATP-linked OCR (30%), Maximal Respiration (111%), Spare Respiratory Capacity (56%), and Non-Mitochondrial OCR (162%)) and in the rates of TCA cycle substrate oxidation, including a 5 fold increase in citric acid oxidation in OXPHOSm. Also, long, medium and short chain fatty acids oxidation was increased, as seen by a large increase in the rate of Acetyl-L-Carnitine oxidation in OXPHOSm.

Conclusions: Our results show that stimulation of oxidative phosphorylation is accompanied by profound remodelling of mitochondrial metabolism, supporting the importance of establishing the appropriate culture conditions to study mitochondrial health or dysfunction.

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54ASM-0400 | Mitochondria as biosensors for disease staging and therapeutic response

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Mitochondria are essential cell organelles, involved in many crucial functions including energy production from the oxidation of different substrates. Many human diseases have been associated with mitochondrial dysfunction either as cause or as consequence. Thus, mitochondrial function may be useful as a source of biomarkers for diagnosis, prognosis and selection of personalized therapeutic strategies. Dermal fibroblasts and lymphoblasts from human donors are useful in precision mitochondrial medicine. However, to evaluate mitochondrial function and to test mitochondria-targeted therapies, some cells need to be cultured in conditions that maintain their metabolic reliance on mitochondria, which is often lost in culture.

To restore cellular reliance on mitochondria, normal human dermal fibroblasts (NHDF) were cultured in no glucose (OXPHOS) conditions, compared to high-glucose (HG) or low glucose (LG), all in the presence of pyruvate and glutamine. Mitochondrial metabolism was assessed using Cell Titer Glo (Promega), confocal microscopy, metabolic phenotypic microarrays (MitoPlates S1 and I1, Biolog) and extracellular flux analysis (Seahorse). NHDF cultured in OXPHOS conditions presented a higher drop in intracellular ATP levels induced by mitochondrial inhibitors, associated with higher OCR, lower ECAR, and increased oxidation of TCA cycle intermediates, fatty acids and lactate, compared to NHDF cultured in HG or LG conditions. Absence of glucose promoted increase in mitochondrial mass in NHDF, associated with increased mitochondrial activity, which are indispensable to study mitochondrial biomarkers of disease and therapeutic response in fibroblasts obtained from human donors.

Previous studies have also shown that alterations in mitochondrial activity and physiology can be detected in lymphoblasts. We analyzed mitochondrial metabolism in lymphoblasts from 6 Amyotrophic Lateral Sclerosis (ALS) patients, 3 of which bearing Superoxide Dismutase 1 (SOD1) mutations (mutSOD1) and 3 without known mutations (nmutSOD1), compared to 3 age- and sex-matched controls, all obtained from Coriell Biorepositories. Results from MitoPlate S1 showed that nmutSOD1 cells had decreased oxidation of glycogen and increased oxidation of glucose-1-PO4 and glucose-6-PO4. These cells seemed to be hypermetabolic, which was confirmed by higher glycolytic ATP production and total ATP production rates, as determined using the Seahorse instrument

These results obtained in different human cell types demonstrate the utility of metabolic phenotypic microarrays to detect cell and mitochondrial metabolic remodeling in the context of human diseases, allowing for the development of novel precision medicine interventions.

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54ASM-0404 | Impact of Rebaudioside A in the metabolism of breast cancer cells and assessment of its chemotherapeutic potential

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Background: Steviol glycosides (SG) are a group of molecules obtained from Stevia, widely used as natural sweeteners. It has been demonstrated that some SG cause a decrease in tumor cell proliferation, accompanied by an increase in reactive oxygen species (ROS) levels and cell cycle arrest. However, the mechanisms are poorly understood. There is few information regarding this for Rebaudioside A (RebA), one of the most relevant SG in the food industry. We studied its impact in human breast cancer cells and human dermal fibroblasts, focusing on viability, metabolic fluxes and oxidative stress.

Materials and Methods: Normal human dermal fibroblasts (NHDF), triple negative breast cancer Hs578t and estrogen/progesterone receptor positive MCF7 cell lines were used. Resazurin and sulforhodamine B assays were performed to evaluate metabolic viability and cell mass, respectively. Combination assays with Doxorubicin (DOX), with either insulin or RebA were also performed, as well as growth curves for those cells in the presence of RebA. Changes in the metabolic profile of tumor and non-tumoral cells were assessed using Nuclear Magnetic Resonance (NMR), Mitoplates S-1 (Biolog) and SeaHorse. Assays using H_2DCFDA were also performed to assess ROS levels.

Results: In the presence of RebA there was a tendency for a small potentiation of DOX toxicity for breast cancer cells, but not for NHDF, while combination with insulin showed no significative differences. Metabolic studies indicated that RebA induces a shift towards an oxidative metabolism, with NMR indicating an increase oxidative phosphorylation fluxes. Mitoplate S-1 assays in NHDF cells suggested that RebA causes a higher rate of consumption of different mitochondrial substrates, including Citric acid, Cis-Aconitic acid

and L-Leucine, indicating increased mitochondrial oxidation rates. By using H2DCFDA as an oxidative stress-sensitive dye, we observed changes in oxidative stress for cells exposed to RebA.

Conclusions: RebA caused a metabolic rewiring towards a more active oxidative phosphorylation, which may be one of the causes for the slight potentiation of Doxorubicin's cytotoxic activity in breast cancer cells. RebA per se has a high IC50 for decreased cell viability, close to its solubility, and, unless used along another drugs, its use in cancer may be limited to lowering the proliferation of tumor cells. This work suggests that the insulin-mimetic effect, in the way that is currently described so far for SG in the literature, may not be sufficient to explain the results and encourages further studies on the use of this sweetener as an adjuvant for chemotherapy or other metabolic therapies.

54ASM-0409 | Glycolytic fluxes and metabolic profiles distinguish amyotrophic lateral sclerosis patients from healthy controls

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Background: Mitochondrial dysfunctions and metabolic alterations contribute decisively to neurodegeneration associated with amyotrophic lateral sclerosis (ALS). Respiratory and glycolytic fluxes, as well as energy metabolism substrate preferences were analysed in lymphoblasts from ALS patients with mutant SOD1 (mutSOD1) or unknown mutations to characterize their mitochondrial profiles.

Materials and Methods: Lymphoblasts from ALS patients with mutSOD1 or unknown mutations, as well as sex/age-matched controls (2 per group) were obtained from Coriell. The groups were composed by one male and one female aged 46 years of each condition. Oxygen consumption (OCR) and extracellular-acidification (ECAR) rates were measured using the Seahorse XFe96 Extracellular Flux-Analyzer. Phenotypic metabolic microarrays were performed using the Biolog MitoPlate S-1 system. Two-way hierarchical clustering and information gain were analysed using Python 3 tools. Data were displayed in box plots. Statistical comparisons were performed by Kruskal-Wallis and Dunn's multiple comparisons test, with P-value<0.05 as significant.

Results: Lymphoblasts from patients with unknown mutation showed higher glucose-6-PO4 and glucose-1-PO4 consumption rates, compared to control ($P < 0.01$, $P < 0.05$) and mutSOD1 ($P < 0.01$, $P < 0.05$), as well as higher values of glycolytic ATP-production rate compared to control ($P < 0.05$), suggesting the activation of glycolysis in patients with unknown mutations. Lymphoblasts with and without known mutSOD1 presented lower oxidation of cis-aconitic acid ($P < 0.01$, $P < 0.05$), and D-L isocitric acid ($P < 0.01$, $P < 0.01$) compared to control; whereas lymphoblasts from patients with unknown mutation also showed decreased oxidation of α-keto-glutaric acid ($P < 0.01$), L-glutamic acid ($P < 0.001$) and glutamine ($P < 0.001$), compared to control. These results suggest that ALS patients with and without known mutSOD1 have different impairments on the tricarboxylic acid cycle. Lymphoblasts from mutSOD1 patients showed lower oxidation of pyruvic acid plus malic acid ($P < 0.05$) and succinic acid ($P < 0.05$) compared to control, as well as lower mitochondrial ATP-production rate when compared to control ($P < 0.05$), an indicator of mitochondrial impairment. A perfect separation between groups was revealed after clustering analysis of the measures with highest information gain (for Seahorse: XF ATP Rate Index, Stressed ECAR, glycolytic and mitochondrial ATP-Rate), with mutSOD1 segregating into a more distinct cluster.

Conclusions: Our results show distinct mitochondrial profiles between mutSOD1 patients and those with unknown mutations, compared to healthy controls, with a better segregation of mutSOD1 patients from controls, compared to those with unknown mutations.

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S3 – CARDIOVASCULAR AND METABOLIC DISEASES

54ASM-0002 | BKca-mediated resveratrol effects in grafts of human saphenous vein from diabetic and non-diabetic patients

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Background: In diabetes mellitus type 2 (DMT2) presence of entire families of smooth muscle potassium channel (K) are changed, limiting vasorelaxation process. As the disease progresses, inadequacy of blood vessels to maintain normal

vascular tone starts to be prominent to the point of losing their integrity, leading to the severe macroangiopathies. Thus, for many patients with already developed complications only treatment option represents CABG (coronary artery bypass-grafting) surgery. On contrary, irreplaceable phytoalexin called resveratrol (RSV) relaxes vascular tissue, acting primarily through different subtypes of K channels, including BKca. We wanted to investigate BKca - mediated RSV relaxation of blood vessel commonly used in CABG - human saphenous vein (HSV) among diabetic and non-diabetic patients.

Materials and Methods: Samples of HSV were taken after CABG surgery of patients with DMT2 and mounted in an organ bath system. Precontracted rings of HSV were relaxed by increasing concentrations of RSV (1-100 μM).

Results: A highly selective BKca-channels blocker, iberiotoxin (100 μM) significantly modified the vasorelaxant effect of RSV on the HSV from patients with DMT2 ($n = 6$ both, $pD2 = 3.8 \pm 0.37$ in the presence vs. 4.8 ± 0.02 in the absence of IbTx, $P < 0.05$), and produced similar response on the HSV from patients without DMT2 ($n = 6$ both, $pD2 = 4.78 \pm 0.04$ in the presence vs. 5.48 ± 0.09 in the absence of IbTx, $P < 0.01$).

Conclusions: Iberiotoxin inhibition of RSV effects in the population of DMT2 and non-diabetic patients is comparable. It seems that BKca channels are preserved in the vascular tissue of DMT2 patients.

54ASM-0032 | Blood clot contraction is impaired in patients with rheumatoid arthritis

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Background: Autoimmune diseases, including rheumatoid arthritis (RA), are the risk factors for thrombotic complications. Contraction (retraction) of blood clots has never been studied in RA, while it can provide new information about hemostatic disorders in RA and inform prevention, prediction, and early diagnosis of the immune thrombosis.

Materials and Methods: An original clot contraction assay was performed in blood samples from 60 RA patients and 50 healthy subjects, using an optical analyzer that automatically tracks the size of blood clots during contraction. Flow cytometry was used to assess the functional state of platelets isolated from the blood of RA patients by the ability to express P-selectin and to bind to fibrinogen.

Results: Clots formed in the blood of RA patients contracted significantly slower and to a lesser extent compared to control group. Changes in the parameters of contraction of blood clots correlated with the laboratory signs of systemic inflammation and were most prominent in the acute stage of the disease. The contraction of blood clots was significantly more suppressed in the RA patients in the active roentgenologic stage of the disease. The impaired clot contraction is most likely caused by defects in platelet functionality that was confirmed by flow cytometry, showing substantial refractoriness of platelets in RA patients in response to stimulation via PAR-1 receptors.

Conclusions: The results obtained confirm the important pathogenic role of hemostatic disorders in RA and support the validity of the blood clot contraction assay as an indicator of pro-thrombotic states. The impaired platelet functionality caused by autoantibodies may be a pathogenic factor that promotes thrombotic complications in autoimmune pathology, including RA.

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54ASM-0035 | Pathogenic antibodies against platelet factor 4/heparin combined with platelet factor 4 cause death and microvesiculation of monocytes

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Background: Heparin-induced thrombocytopenia (HIT) is an autoimmune disorder caused by heparin treatment and characterized by the presence of anti-platelet-factor 4(PF4)/heparin antibodies (HIT-Abs) in the blood associated with thrombocytopenia and arterial or venous thrombosis. HIT-Abs react with a complex of PF4 with glycosaminoglycans on the surface of endothelial and blood cells, including monocytes. Here, we investigated effects of the mixture of PF4 and pathogenic HIT-like Abs (named KKO) on human monocytes.

Materials and Methods: CD14-positive monocytes of >85% purity were isolated from citrated blood of healthy donors using negative immune-magnetic separation. Monocytes were incubated without or with a mixture of pure PF4 and pathogenic KKO or control non-pathogenic anti-PF4/heparin monoclonal Abs. Viability of monocytes was determined with flow cytometry by expression of phosphatidylserine

(Annexin V binding) and cell penetration for propidium iodide (PI). To identify membrane-derived microvesicles, monocytes were pre-incubated with a membrane stain (DiI) and treated with Triton X-100 to destroy the particles made of phospholipids.

Results: The largest fraction of monocytes (13%) expressing phosphatidylserine and permeable for PI (Annexin-V/PI-positive) was after treatment with PF4/KKO, while control cells (untreated or treated with PF4 alone or control Abs) contained only 2-9% of Annexin-V/PI-positive monocytes. The number of DiI/CD14/Annexin-V-positive microvesicles increased on average by 50% in the PF4/KKO-treated cell preparations (18 microvesicles /µl) compared with the untreated cells (12 microvesicles /µl) or PF4-treated monocytes (9 microvesicles /µl). The microvesicles were not detectable after incubation of cells with Triton X-100, indicating their membrane-derived lipid nature.

Conclusions: Interaction of monocytes with a combination of PF4 and pathogenic HIT-like Abs causes death of monocytes associated with release of phosphatidylserine-expressing microvesicles. The growing population of dying monocytes and formation of procoagulant microvesicles in the blood of HIT patient may cause and/or exaggerate the prothrombotic status.

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54ASM-0040 | Right Ventricular Dysfunction in Cancer Patients Treated with Anthracyclines. Are beta-blockers of help in prevention?

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Background: We evaluated the right ventricular diastolic and systolic performance in cancer patients treated with epirubicin, using Doppler echocardiography.

Materials and Methods: 60 patients with different malignant tumors treated with epirubicin up to 500 mg/m² and beta-blockers (study group), and a gender- and age-matched group of 60 patients diagnosed with tumors, on treatment with epirubicin (control group), were assessed by echocardiography. Patients with associated cardiac diseases affecting the ventricular diastolic function were excluded. We assessed the right ventricular diastolic function by measuring the Doppler transtricuspidian flow: the maximal velocity of the E and A wave, the ratio of Emax/Amax, the pressure half time (PHT) of the E wave. We also assessed the right ventricular systolic performance (volumetric method).

Results: We documented an alteration of the right ventricular diastolic performance in both groups, with a significant decrease ($P < 0.01$) of Emax, whereas the A wave was significantly increased ($P < 0.01$), the E/A ratio becoming sub-unitary. We also documented a prolonged PHT of the E wave, after completion of chemotherapy ($P < 0.01$) in both groups. Associated treatment with beta blockers in the study group, failed to prevent the alteration of the right ventricular diastolic performance. The right ventricular systolic performance remained in normal range in both groups, after completion of chemotherapy.

Conclusions: Epirubicin chemotherapy in cancer patients, deteriorates the right ventricular diastolic performance, and associated treatment with beta-blockers could not prevent this alteration.

54ASM-0048 | Composition and structure of cerebral thrombi correlated with their intravital contraction and clinical features of acute ischemic stroke

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Background: Acute ischemic stroke remains one of the leading causes of morbidity and mortality worldwide. The structure and composition of thrombi must have a strong relation to clinical manifestations of stroke.

Materials and Methods: Using scanning electron microscopy and histology, we analyzed qualitatively and quantitatively the structure and composition of fresh cerebral thrombi extracted from 40 patients with acute ischemic stroke and correlated variations in the thrombi composition with etiology, severity, duration and outcomes of stroke.

Results: Cerebral thrombi were made of blood cells and fibrin and had very low porosity. The prevailing cell type was compressed polyhedral erythrocytes (polyhedrocytes), indicative of intravital contraction. The content of polyhedrocytes directly correlated with the stroke severity. The presence of fibrin bundles

over single fibers was typical for more severe cases, while the content of fibrin sponge prevailed in cases with a more favorable outcome. The higher content of platelet aggregates was another marker of stroke severity, although the overall platelet content in these arterial thrombi was unexpectedly small. Fibrillar types of fibrin dominated over fibrin sponge in atherothrombogenic thrombi. Cerebral thrombi with a substantial number of leukocytes, often arranged into clusters, were usually associated with fatal cases. Histologically, the majority of thrombi also had pronounced morphological signs of contraction, including polyhedrocytes in the core and redistribution of fibrin to the periphery. Thrombus age was assessed by histological staining of fibrin, with older fibrin present in thrombi from the patients who received thrombolytics and younger fibrin prevailing in cardioembolic thrombi. Alternating layers of erythrocytes and fibrin/platelets were typical for thrombi from the patients with more favorable outcomes.

Conclusions: The composition of cerebral thrombi is variable and generally correlates with the clinical course and outcomes of acute ischemic stroke.

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54ASM-0064 | Anxiety and depression in patients with type 2 diabetes mellitus

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Background: Depression is an urgent general medical problem. In type 2 diabetes mellitus (DM-2) it leads to poor glycemic control and increases the risk of complications. Objective: to assess the level of anxiety and depression (A&D) in patients with DM-2.

Materials and Methods: Inclusion criteria: patients with DM-2; age 40-75 years; glomerular filtration rate over 60 mL/ min/1.73 m^2. Exclusion criteria: impaired sensations, pain and decreased strength in the limbs. A&D was assessed by the Hospital Anxiety and Depression Scale (HADS).

Results: 120 patients with DM-2 (48 men and 72 women, average age 61.22 ± 8.6 years). Average duration of the disease:10.84 ± 8.2 years. Blood glucose level: 5-9 mmol/l, HbA1C level: 5-6.5%. Average HADS results: depression – 7 [4;10] points, anxiety – 7 [5;11] points (subclinical depression and anxiety). Depression scale results – 70 patients (58.3%): 0-7 points (normal), 34 patients (28.3%): 8-11 points (subclinical depression), 16 patients (13.3%): >11 points (clinically severe depression). Anxiety scale results – 62 patients (51.7%): 0-7 points (normal), 32 patients (26.7%): 8-11 points (subclinical anxiety), 26 patients (21.7%): >11 points (clinically expressed anxiety). The study revealed positive correlation of HADS results with the duration of DM-2 and patients' age ($P < 0.05$); the absence of correlation of HADS results with the level of glycaemia ($P > 0.05$)

Conclusions: In DM-2, possible mechanisms for the development of A&D include metabolic disturbances in the brain, psychological stress, and insulin resistance of neurons, which causes dopaminergic dysfunction. The average A&D indicators in patients with DM-2 are subclinical, but statistically significantly higher than in the general population ($P < 0.0001$). A&D is an important comorbid state of DM-2. It develops in more than 50% of DM-2 cases, influences negatively the course of the disease, aggravates neurodegeneration and decreases the quality of life.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

54ASM-0094 | The effect of cord blood cell transplantation and curcumin administration on plasma HexA activity and serum cytokine profile in Tay-Sachs disease patient

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Background: Tay-Sachs disease (TSD) is an autosomal recessive disorder belonging to the group of lysosomal storage diseases which leads to acute neurodegeneration as a result of GM2-ganglioside accumulation inside nerve cell lysosomes due to a deficiency of β-hexosaminidase A (HexA). Cord blood cell transplantation (CBCT) can delay neurodegeneration in TSD patients, in turn, curcumin increases HEXA expression in fibroblasts *in vitro.*

Materials and Methods: In this study we evaluated the effect of CBCT and curcumin administration on plasma HexA activity and serum cytokine profile in TSD patient. Plasma and serum of healthy donors (n = 10) were used as a control. Plasma HexA activity was evaluated using a MUGS fluorescent substrate. Serum cytokine profile was determined using immunology multiplex assay (41-plex, Merk). The patient took curcumin for 3 months according to the following regime: 500 mg/day for 10 days, 1000 mg/day for 10 days and 3500 mg/day for 65 days.

Results: It was shown that CBCT and curcumin administration did not influence HexA activity in blood plasma of TSD patient which remained much lower compared to healthy donors. Multiplex analysis showed that the levels of IL-8, sCD40L, Eotaxin, VEGF, EGF and MDC in TSD patient

serum were significantly increased compared to the control group. However, after CBCT IL-8, sCD40L and EGF levels were not statistically different in comparison with healthy donor levels. A decrease in IP-10, Eotaxin, VEGF, PDGF-AA and EGF levels after curcumin administration was also observed.

Conclusions: Thus, the obtained data may indicate that CBCT and curcumin administration do not directly affect HexA activity, and however, it can reduce CNS inflammation which is usually observed in TSD patients. This work was funded by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities and by the Russian Government Program of Competitive Growth of KFU.

54ASM-0147 | Obesity-related transcripts abundance in sperm and clinical success of assisted reproduction

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Background: Obesity-related genes (ORG) have been pointed out as causes for an overweight/obese phenotype, but their relevance to sperm quality and embryo development remains unknown. In this work, we hypothesize that ORG, specifically, melanocortin-4 receptor (*MC4R*), Fat mass and obesity (*FTO*), and Glucosamine-6-phosphate deaminase 2 (*GNPDA2*) are present in human spermatozoa and are associated to sperm quality and embryo development after treatment with assisted reproduction techniques (ART).

Materials and Methods: This study was based on a cohort of 106 couples seeking for fertility treatment. Quantitative polymerase chain reaction (qPCR) was used to access ORG transcript abundance in spermatozoa. Sperm quality parameters were evaluated by standard spermogram according to WHO guidelines. Embryo quality and pregnancy evolution were evaluated by a certified embryologist at the fertility clinic. Protein expression of these ORG was evaluated by Western blot (WB) and Immunofluorescence staining (IF).

Results: We identified for the first time the presence of *MC4R* and *GNPDA2* transcripts and respective proteins in spermatozoa, through PCR, WB and IF. Although paternal age and body mass index were not correlated with the abundance of ORG transcripts in spermatozoa, our data show that abundance of *MC4R* and *FTO* transcripts was correlated with sperm viability ($q = -0.3111$) and total sperm count ($r = 0.5042$), respectively. The abundance of *FTO*, in spermatozoa, was also correlated with the fertilization-rate ($r = 0.4751$), embryo cleavage rate ($r = 0.6530$) and high-quality embryo rate ($r = 0.6544$), during ART. *MC4R* abundance in spermatozoa was correlated with biochemical pregnancy ($r = 0.4502$), a parameter associated with the embryo implantation.

Conclusions: Overall our data show that abundance of ORG transcripts in spermatozoa is associated to relevant parameters of sperm quality and the clinical success of ART. Further studies will be needed to unveil the role of these ORG in the male gamete and its implications for the reproductive event.

54ASM-0187 | Triple gene therapy for the prevention of ischemic stroke

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Background: Currently, to overcome the consequences of ischemic stroke gene and cellular engineering technologies are widely employed. Recently, we showed that delivery of genes encoding vascular endothelial growth factor (VEGF), glial cell-line derived neurotrophic factor (GDNF) and neural cell adhesion molecule (NCAM) using human umbilical cord blood mononuclear cells (UCBC) effectively prevents neurons death and has a positive effect on brain remodeling in rats after middle cerebral artery occlusion (MCAO).

Materials and Methods: In the present study we employed the same combination of genes for preventive gene therapy of ischemic stroke in rats. For direct gene delivery animals were intrathecally injected with a mixture of three adenoviral vectors (Ad5) carrying human genes (2×10^7 Ad5-VEGF+Ad5-GDNF+Ad5-NCAM). For cell-mediated gene delivery rats were infused with 2×10^6 UCBC, carrying the same gene constructs. Animals in the control group 3 days before MCAO received saline.

Results: Three and four days after direct or cell-mediated gene delivery, respectively, MCAO was performed in all experimental rats. Three weeks after MCAO morphometric analysis of the infarction focus in the parietal cortex

showed that in rats after preventive gene therapy the infarct volume was significantly less than in control animals. Immunofluorescence analysis of the peri-infarct area found that the number of caspase-3-positive cells was significantly lower in rats after preventive gene therapy, when compared with control animals. At the same time, the level of Hsp70 immunoexpression in rats after gene therapy was significantly lower than in the control animals.

Conclusions: Our results suggest that intrathecal infusion of UCBC overexpressing VEGF, GDNF, and NCAM or simultaneous injection of three Ad5 carrying the same genes before vascular catastrophe significantly increases the survival of neurons and reduces the infarct volume with the onset of stroke.

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54ASM-0206 | Evaluation of matrix metalloproteinase-11 expression under conditions of insulin resistance

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Background: Insulin resistance (IR) is associated with obesity and type 2 diabetes. The matrix metalloproteinase-11 (MMP-11) is a proteinase involved in remodeling of extracellular matrix (ECM). By adopting an *in vitro* and *in vivo* model of IR, we planned to investigate the expression and the secretion of MMP-11.

Materials and Methods: 3T3-L1 cells were differentiated into adipocytes and were made IR by hypoxia and/or TNF-α treatment. Mass spectrometer was used to analyze the proteins secreted by IR cells. Mice were fed with low or high fat diet to induce insulin resistance.

Results: The expression of MMP-11 was assessed during 3T3-L1 differentiation into adipocytes. MMP-11 mRNA abundance was low in 3T3-L1 pre-adipocytes, increased in confluent cells and reached maximal expression in mature 3T3-L1 adipocytes. Afterwards, MMP-11 levels were measured in IR adipocytes. A significant increment of mRNA and protein expression was observed in the IR model compared to normal cells. An increase in MMP-11 expression was demonstrated also in the presence of TNF-α or hypoxia alone. The analysis of the secretome of 3T3-L1 adipocytes, exposed to either normoxia or hypoxia, showed a significantly down regulation of adipokines, such as adiponectin, thrombospondin-1 and thrombospondin-2, and MMP-11. Results obtained *in vitro* were confirmed in the IR animal model. In particular, MMP-11 mRNA was upregulated in white adipose tissue (WAT) from obese mice compared to lean mice. In addition, in obese animals, the increment of MMP-11 expression was accompanied by the increase of typical markers of fibrosis, such as collagen type VI alpha 3 (col6α3) and fibroblast-specific protein 1 (FSP-1)

Conclusions: Our data demonstrate that the dysregulation of MMP-11 expression may be an early trigger for ECM remodeling and adipose cell impairment leading to obesity-related IR. Furthermore, secreted MMP-11 could serve as an early biomarker of adipose tissue dysfunction.

54ASM-0210 | Involvement of septins in platelet function

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Background: Platelets are the smallest blood cells that are important in hemostasis and thrombosis. Shape changes of activated platelets are associated with the reorganization of cytoskeleton. Septins, a GTP-binding and membrane interacting cytoskeletal proteins, are involved in various cellular processes including cytokinesis and mitosis of nucleated cells, but their role in platelets has not been addressed.

Materials and Methods: By using a combination of confocal microscopy, flow cytometry, biomechanical and biochemical assays we examined how septins are involved in structural and functional alterations in human activated and contracting platelets. Septins 2 and 9 were immunostained in platelets, either spread on a fibrinogen-coated surface or contracting within a 3D plasma clot. Clot contraction was evaluated by tracking the clot size.

Results: In resting platelets, septin 2 concentrated at the cell periphery, colocalizing with the microtubule marginal ring, while septin 9 was distributed as small patches over the cell volume, often with a peripheral localization. In thrombin-activated platelets, septins were also observed in filopodia. Activation of platelets resulted in a 2-fold increase of septins intensity. Inhibition of septins dynamics with forchlorfenuron (FCF) caused dose-dependent perturbations, including a substantial decrease of platelet roundness and surface curvature. In FCF-pretreated platelets stimulated with TRAP, expression of activated integrin αIIbβ3 was significantly suppressed. FCF impeded clot contraction with a 6-fold increase of the lag-time and up to a 3-fold decrease of the extent of contraction. Alterations in septin structure caused

by FCF abolished platelet spreading by 50% and accelerated thrombin-induced platelet fragmentation.

Conclusions: Septins are important for stabilizing platelet shape and supporting platelet integrity; septins are involved in platelet biomechanical functions, such as contractility and adhesion.

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54ASM-0234 | Lipoxin profiles in human acute heart failure and cardiogenic shock and their modulation by aspirin or statin therapy

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Background: The specialized proresolving mediators lipoxin A_4 (LXA_4) and 15-epi-LXA_4 were previously shown to be reduced in human severe chronic heart failure (CHF). Here, we evaluated the serum and urinary LXs profiles in patients with acute heart failure (AHF) and cardiogenic shock (CS). Moreover, the impact of aspirin or statin treatment on 15-epi-LXA_4 values was also analyzed.

Materials and Methods: Patients with AHF (n = 12) or CS (n = 13) were included and blood and urine samples were collected at days 1-2, days 3-4 and days 5-7. There were 6 AHF and 4 CS patients on aspirin (ASA) treatment and 8 AHF and 6 CS patients on statin treatment. Blood donors were used as controls (n = 10). Serum (s) and urinary (u) LXA_4 and 15-epi-LXA_4 were determined by ELISA kits.

Results: At admission, we found no differences in sLXA_4 or s15-epi-LXA_4 values between groups. In contrast, both uLXA_4 and u15-epi-LXA_4 (ng/mg creatinine) significantly increased in AHF patients (uLXA_4, controls: 0.50 ± 0.16; AHF:1.80 ± 0.35; CS:0.68 ± 0.17, controls vs AHF, $P = 0.001$; AHF vs CS, $P = 0.01$, controls vs CS, P = ns; u15-epi-LXA_4, controls: 1.44 ± 0.66; AHF:4.84 ± 1.41; CS:4.33 ± 1.83, controls vs AHF, $P = 0.004$; CS vs controls or vs AHF, P = ns). During hospitalization, sLXs significantly decreased in both patients' groups. From day 3 to 7, AHF but not CS patients treated with aspirin had higher mean values of s15-epi-LXA_4 ($P = 0.035$) and tendentially higher mean values of u15-epi-LXA_4 ($P = 0.057$) compared to patients without aspirin therapy. In contrast, AHF but not CS patients on statin therapy tended to have lower s15-epi-LXA_4 ($P = 0.055$) compared to patients without statin treatment. Moreover, when comparing patients treated only with aspirin or only with statin, aspirin treatment was associated with higher s15-epi-LXA_4 concentrations ($P = 0.048$).

Conclusions: AHF episodes are associated with an acute increase of uLXs at hospital admission. Noteworthy, aspirin but not statin treatment appears to favour 15-epi-LXA_4 production in AHF. [Funded by FCT/FEDER-COMPETE, Portugal 2020-PTDC/MEC-CAR/32188/2017]

54ASM-0256 | Multi-parametric analysis of blood pressure and pulse intervals mutual dynamics during tilt test

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Background: Multi-parametric analysis of blood pressure and heart rate coupling during tilt test provides significant information underlying the cardiovascular system regulation including arterial baroreflex disorders.

Materials and Methods: We analyzed the systolic blood pressure (SBP) and pulse intervals (PI) records of three participant groups: 33 patients with non-diabetic moderate autonomic dysfunction; 25 patients with diabetes mellitus; and 28 healthy volunteers. We studied six indicators of SBP and PI mutual dynamics: (i) the phase synchronization coefficient *Sync*, (ii) the averaged correlation time of the phase differences of SBP and PI data series *TAU*, (iii) the time delay stability coefficient *TDS*, (iv) the averaged coherence *Coher*, (v) the cross-conditional entropy *CE*; and (vi) the baroreflex sensitivity index *BRS*. All indicators were determined both from the entire tilt test records as well as from each of either supine or orthostatic tilt test phases.

Results: All analyzed indicators significantly differed between the patient groups and the control group containing healthy volunteers ($P < 0.05$) both for the entire record as well as for separate the tilt test phases. The phase synchronization coefficient *Sync*, the time delay stability coefficient *TDS* and the baroreflex sensitivity index *BRS* also differed significantly between two patient groups ($P < 0.05$) thus allowing for their differential diagnostics. For better visualization of the differences between the indicators in all studied groups during all the tilt test phases visualization in the form

of star-style diagrams depicting the normalized indicators where each of the six coordinate axes corresponds to one of the studied metrics. Filled areas represent the interquartile ranges and with ticks marking their medians.

Conclusions: Our results indicate that the addition of complementary coefficients such as the phase synchronization coefficient *Sync* and the time delay stability coefficient *TDS* to the traditionally used baroreflex sensitivity index *BRS* could be beneficial for the improvement of the differential diagnostics of blood pressure regulation efficacy in patients with various autonomic disorders.

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54ASM-0257 | The heart rate peak value prediction during physical stress test

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Background: Physical stress test is a most common non-invasive, cheap and reproducible method for the human cardiovascular system diagnostic. However, there is a serious problem that the exercise during such test is physically difficult for a person. Predicting the functional state of patients at the end of a stress test protocol based on data observed only at the first stages of an exercise is an important point of such diagnostic.

Materials and Methods: In this study 149 anonymized R-R interval sequences in patients with congestive heart failure (97 men and 52 women) were used. These patients performed the exercises on a bicycle ergometer with a step load protocol at Almazov National Medical Research Center, Saint Petersburg. And 29 anonymized R-R interval sequences in patients with ischemic heart disease (19 men and 10 women) obtained at the same conditions at Pavlov First St. Petersburg State Medical University.

Results: An empirical linear regression was obtained to estimate the peak heart rate value. It was shown that the regression coefficients vary significantly from the patient's gender. The relative prediction error for the HR peak value did not exceed 10%. It was also shown that for patients with congestive heart failure and ischemic heart disease the quadratic dependence is adequate model for describing the dynamic of the HR during the stress test.

Conclusions: This study is the first step to reducing the duration of the stress test without loss of diagnostically relevant information by processing the data obtained in the first stages of the test.

54ASM-0267 | Proinflammatory cytokines and endothelial cell activation in human acute heart failure and cardiogenic shock

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Background: Inflammation-driven 'endothelitis' appears to contribute to acute heart failure (AHF). We aimed at evaluating the proinflammatory status and endothelial activation, as well as their correlation, in human AHF and cardiogenic shock (CS).

Materials and Methods: Patients with AHF (n = 23) or CS (n = 25) were included and blood samples were collected at days 1-2, days 3-4 and days 5-8. Blood donors were used as controls (n = 22). Serum IL-1β, IL-6, tumoral necrosis factor-α (TNF-α), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin were determined using Multiplex Immunoassays.

Results: At admission, we found no significant differences in IL-1β concentration between groups. Admission IL-6 values (pg/mL) were higher in both patients' groups (controls:2.0 ± 0.9; AHF:18.1 ± 4.4; CS:95.9 ± 26.4, controls vs AHF or CS, $P < 0.001$). There was an overall significant difference in TNF-α concentration (pg/mL) between groups ($P = 0.03$), with patients' groups exhibiting tendentially higher values (controls:5.4 ± 0.6; AHF:11.9 ± 3.1; CS:9.4 ± 1.5, controls vs AHF, $P = 0.06$; controls vs CS, $P = 0.07$). Admission ICAM-1 values (ng/mL) were increased in CS compared to controls and AHF (controls:474.9 ± 70.7; AHF:329.7 ± 19.7; CS:665.5 ± 72.6, controls vs CS, $P < 0.05$; AHF vs CS, $P = 0.001$). Admission VCAM-1 (ng/mL) was higher in both patients' groups (controls:705.8 ± 51.0, AHF:1482.0 ± 161.7, CS:3425.0 ± 645.6, controls vs AHF, $P < 0.01$; controls vs CS, $P < 0.001$), while E-selectin did not differ between groups. During hospitalization, there was an overall increase in IL-1β and ICAM-1 values ($P = 0.009$ and $P = 0.04$, respectively) in AHF. Within

patients, we observed significant positive correlations between proinflammatory cytokines and endothelial markers, as well as within both groups of biomarkers.

Conclusions: Proinflammatory status and endothelial cell activation are interrelated and significantly increased in both patient's groups. During hospitalization, the significant rise in ICAM-1 and IL-1β values, along with the lack of significant changes in IL-6 and VCAM-1, suggests that AHF treatment is not sufficient to counteract inflammation-driven 'endothelitis' which might perpetuate and aggravate heart failure. [Funded by FCT/FEDER-COMPETE, Portugal 2020-PTDC/MEC-CAR/32188/2017]

54ASM-0305 | Type 2 diabetes mitigates brain cortical energy metabolism in middle-aged female rats

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Background: Type 2 diabetes (T2D) augments the risk for neurodegenerative events, such as Alzheimer's disease (AD). The pathophysiological mechanisms shared by both disorders may be further modulated by female sex (especially during perimenopause and menopause). Hence, we hypothesized that sex differently impacts T2D brain metabolic function and, consequently, the susceptibility to AD at midlife.

Materials and Methods: We aimed to analyze the role of sex on brain cortical energy metabolism in middle-aged T2D rats. We used brain cortical homogenates from middle-aged (8-month-old) male and female Wistar and non-obese T2D Goto-Kakizaki (GK) rats to evaluate markers for glucose and mitochondria-related energy metabolism, by HPLC and colorimetry.

Results: Despite the markedly higher glycemia, brain glucose levels only slightly increased in T2D female rats compared to GK males. Brain glucose levels were massively decreased in Wistar females, pointing towards an impairment in their brain glucose transport and/or in its downstream metabolism. Brain pyruvate levels and citrate synthase activity were tendentiously decreased in both control and T2D females, suggesting an attenuation of brain glycolysis and early stage of Krebs cycle. In spite of the overall inhibition of brain cortical mitochondrial complexes I-IV in middle-aged T2D female brains, ATP pool was not affected.

Conclusions: Mitigation of brain cortical energy metabolism in middle-aged T2D females may account for their lower accumulation of AD-like hallmarks. Nonetheless, further studies are needed to clarify the precise crosslinking mechanisms.

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54ASM-0325 | Predictability of premature ventricular complexes

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Background: A series of consecutive premature ventricular complexes (PVCs) can be considered as a sequence of events that occur either due to pathophysiological process development or due to randomly occurring physical and psycho-emotional stress factors. Here we analyze the sequence of intervals between consecutive PVCs in terms of their probability density functions (PDF) and autocorrelation functions (ACF) and show how their knowledge could contribute to the predictability of the next PVCs occurrences.

Materials and Methods: 18 24-hour Holter recordings from the MIT-BIH Long-Term Database and the ST Long-Term Database were used for the analysis. The recordings containing at least 1000 PVCs were selected to obtain reliable statistics and sequences of time intervals τ between consecutive PVCs. We found that the PDF decays by a power law $P(\tau)\sim\tau^{-\delta}$, where $\delta>1$, for $\tau>6$ seconds. For the intervals between the clusters of consecutive PVCs we found that the ACFs also decays by a power law $C(s)\sim s^{-\gamma}$ also, where $\gamma=2-2H$, H is the Hurst exponent. The estimations of H were obtained using detrended fluctuation analysis (DFA) method for the considered data set, and typically fall in the range from 0.62 to 0.87. Since long-term correlations in the PVC cluster sequence could be observed, it gives rise to their

predictability. In the following, we considered five different methods for PVCs prediction.

Results: We considered the simple averaging method, the conditional averaging method, the autoregressive (AR) method, the optimal linear forecasting and the proposed non-linear forecasting method, which we call the 'triples' method. To compare the efficacy of forecasts, the mean square of the prediction error (D_e) and parameter $P = 1\text{-}D_e/D_T$ have been used, where D_T is the variance of the analyzed time intervals. The best result was achieved with the optimal linear forecasting method. Close results were shown by the AR method and the 'triples' method. All the considered methods are characterized by relatively low forecasting accuracy characterized by $P \leq 0.38$.

Conclusions: Our results indicate that the PVC clusters in general could be predicted, although the information extracted from the preceding interval sequences appears insufficient to obtain reliable predictions, while further improvements might be achieved by using additional physiological status indicators.

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54ASM-0337 | Diet-induced high cardiovascular risk compromises brain hippocampal metabolism and long-term spatial memory in middle-aged male rats

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Background: High cardiovascular disease (HCR) is the leading cause of disability and death in middle-aged men. It comprises obesity, hypertension, dyslipidemia, diabetes – all aging-/lifestyle-associated diseases, that worsen its prognosis and increase the risk for cognitive impairment and Alzheimer's disease (AD). Still, the crosslinking mechanisms involved are unknown. We hypothesized that HCR at midlife affects brain metabolism and cognitive performance predisposing to AD.

Materials and Methods: We aimed to analyze the impact of HCR on brain metabolism, cognition and accumulation of AD-like features in middle-aged male spontaneously-hypertensive (SHR) rats and in rats fed on salted diet. We evaluated peripheral features of HCR and hippocampal-related spatial memory, alongside the colorimetric measurement of cAMP, cholesterol and phospho-Tau levels in hippocampal lysates.

Results: Rats fed on salted diet had higher blood pressure, insulin levels and HOMA-IR suggesting that there were hypertensive and insulin resistant. Their body weight, glycemia, HbA_{1C} and total cholesterol was similar to SHR rats, suggesting that they were at HCR. Moreover, these HCR rats took longer to reach the Morris watermaze platform, crossed less the swimming-pool, spent less time in the right quadrant and more in the opposite one, suggesting a deficit in their long-term, hippocampal-related spatial memory. HCR rats also exhibited lower hippocampal cAMP, cholesterol and phospho-TauThr181 (related with earlier AD stages) than SHR rats.

Conclusions: Diet-induced HCR impairs middle-aged male rat brain metabolism and long-term spatial memory. However, this issue deserves further clarification.

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54ASM-0355 | Direct oral anticoagulants in patients with cardiac implantable devices and non-valvular atrial fibrillation: the complexity of dose adjustments and the impact on outcomes

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Background: Direct oral anticoagulants (DOACs) are the first line therapy for stroke prevention in non-valvular Atrial Fibrillation (NVAF). Observational studies evidence widespread discordance between guidelines and real-world practice regarding DOACs doses. Moreover, the use of off-label doses is related to an increased risk of stroke, bleeding and/ or adverse effects. This real-world population study aimed to evaluate the complexity of DOACs dose adjustment in Portuguese patients with cardiac implantable electronic devices (CIEDs) and assess the impact of using off-label doses in safety and efficacy outcomes.
Materials and Methods: A single-centre observational retrospective study was performed enrolling all 220 NVAF patients implanted with a CIED between January 2011 and February 2013 in Cardiology Department of Coimbra Hospital and Universitary Centre that are taking DOACs during the study time. This study was approved by local Ethics Committee. Considering DOACs switch, 314 dose adjustments were considered for analysis. Baseline demographic and clinical characteristics were evaluated, and the dose adjustments were assessed according with EMA guidelines.
Results: In this cohort, mean age was 82.1 ± 8.0 years and 65.4% of the patients were male, with a mean body mass index of 28.2 kg/m^2. Regarding the specific DOAC, 36.6% received dabigatran, 29.0% rivaroxaban, 29.0% apixaban, and 5.4% edoxaban. Evaluation of dose adjustments demonstrated that if they were based only in renal function, only 47.1% of the patients were correctly adjusted. However, when all the data were integrated (weight, age, concomitant drugs and increased bleeding risk), 67.8% of the dose adjustments were according to European guidelines. Approximately 32.2% of the patients were administered with off-label doses: 22.3% of them received underdosed off-label doses while 9.9% were overdosed. Edoxaban was the DOAC most frequently overdosed while rivaroxaban was the most frequently underdosed. In addition, 55 complication events, such as stroke, bleeding events and myocardial infarction were identified. Among them, 44.4% were ischemic and 55.6% hemorrhagic. Furthermore, 12 of the 55 events (21.8%) were identified in patients taking off-label doses of DOACs. Also, from the 6 events reported in overdosed patients, 4 were hemorrhagic.
Conclusions: A significant proportion of NVAF patients with implantable devices under DOAC therapy were administered with off-label doses which can lead to an increased risk of adverse effects. In fact, more than one fifth of the events identified occurred in patients taking off-label DOAC doses. The fact that regulatory agencies, guidelines and drug labelling are worldwide different hamper the adjustment of DOACs doses in clinical practice.

54ASM-0401 | The impact of high-fat feeding on direct and indirect pathway contributions to hepatic glycogen synthesis in a mouse model of non-alcoholic fatty liver disease

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Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is considered the hepatic manifestation of the metabolic syndrome. In 2018, approximately 25% of the worldwide population was estimated to suffer from NAFLD and the incidence rates are expected to increase. NAFLD is characterized by an imbalanced triglyceride uptake and clearance, leading to excessive hepatic fat accumulation. This is associated with the development of hepatic insulin resistance, which in turn disrupts additional metabolic processes that are dependent on insulin signaling, including postprandial glycogen synthesis. Glycogen synthesis from glucose via the direct pathway, mediated by glucokinase, is more dependent on insulin actions compared to synthesis via the indirect pathway. We, therefore, hypothesized that high-fat feeding reduces the direct pathway contribution to glycogen synthesis relative to that of the indirect pathway.
Materials and Methods: Over a 16-week period, 12 C57/ BL6 male mice were fed a high-fat diet (HF) and a control group of 9 male mice was fed standard chow (SC). At the start of the final evening, deuterated water (^2H_2O) was administered. Afterward, they were allowed to feed ad libitum overnight and were then euthanized the following morning. Livers were freeze-clamped, and glycogen was extracted and digested to glucose, which was then derivatized to monoacetone glucose (MAG) and purified for ^{2}H-NMR analysis.

Preliminary uncorrected estimates of percent direct and indirect pathway contributions to hepatic glycogen synthesis were made by measuring ^{2}H enrichment of glycogen position 5 relative to position 2.

Results: For Standard Chow mice, the direct pathway contributed 54.73 ± 6.15% of overnight glycogen synthesis with 45.27 ± 6.15% originating from the indirect pathway. High-fat mice had significantly lower direct pathway contribution (23.70 ± 7.06%, $P < 0.05$) and correspondingly higher indirect pathway fractions (76.30 ± 7.06%, $P < 0.05$).

Conclusions: These data indicate that hepatic glycogen synthesis was remodeled by high-fat feeding in a manner that is consistent with the development of hepatic insulin resistance.

54ASM-0417 | The neuroprotective effects of peripheral treatment with Evolocumab in middle-aged rat at high cardiovascular risk

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Background: Healthy lifestyle and diet may prevent cardiovascular disease and brain dysfunction. Conversely, high-fat and/or salted diet increase the risk for cardiovascular disorders, brain/cognitive impairment and ultimately Alzheimer's disease (AD). Thus, the efficient lipid-lowering monoclonal antibody against PCSK9 Evolocumab used to treat dyslipidemia may mitigate such brain/cognitive changes upon high cardiovascular risk (HCR). We hypothesized that Evolocumab protects the brain from HCR rats at midlife.

Materials and Methods: We aimed to analyze the effects of subcutaneous (s.c.) Evolocumab in markers for HCR, AD and neurodegeneration/death mechanisms in diet-induced HCR rats. We used blood and brain lysates, and colorimetry/fluorimetry approaches to assess those parameters.

Results: HCR rats presented hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia and diabetes. Evolocumab treatment reduced their blood cholesterol levels. This was accompanied by a slight reduction in their brain phosphorylated Tau protein at Ser396 (known to be phosphorylated in medium-late stage of AD) and in the activities of caspases-1 and −12 upon s.c. Evolocumab that, nonetheless, was accompanied by a tendentious stimulation of caspase-3. These results suggest that s.c. Evolocumab may partially protect against the inflammation- and/or endoplasmic reticulum stress-associated apoptosis, and the accumulation of neuropathological markers of AD.

Conclusions: Evolocumab may be a neuroprotective therapy against brain dysfunction associated with HCR. However, this issue deserves further clarification.

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S4 – HEALTHY CHILDREN FOR HEALTHY ADULTS: TRANSLATING RESEARCH INTO LIFESTYLE

54ASM-0063 | Central sensitization in adolescents with migraine and tension-type headache

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Background: Central sensitization is a wide range of states, including musculoskeletal, gastrointestinal and nervous system dysfunction, combined with pain and chronic fatigue. Central sensitization (CS) has a negative effect on the course and progression of pain syndromes in adolescents. CS can occur in adolescents with migraines, tension-type headaches (TTH), benign joint hypermobility syndrome (BJHS), and

juvenile fibromyalgia. Objective of the study: to assess CS in adolescents with migraine and TTH.

Materials and Methods: The study included 78 adolescents divided into two groups: group 1 (G-1) – patients with migraine (36 people) and group 2 (G-2) – patients with TTH (42 people). All patients passed through the standard neurological examination and kept headache diaries. CS was evaluated by the validated Russian-language version of Central Sensitization Inventory. Joint hypermobility was assessed using Brighton criteria.

Results: The average age of patients: G-1 = 13 [13;16] years, G-2 = 14 [14;15] years ($P = 0.8$). The number of days with a headache per month: G-1 = 10 [5;15], G-2 = 10 [8;15] days ($P = 0.64$). Adolescents with migraine suffered from headaches for a longer time (60 [36;108] months) than adolescents with TTH (24 [12;48] months). However, CS was more significant in patients with TTH than in patients with migraine ($P = 0.03$). According to Brighton criteria, CS has a higher level of severity in patients without BJHS than in patients with BJHS (37 [27;43] and 24 [21;35] points, respectively).

Conclusions: TTH is associated with a greater severity of CS, so during the treatment it is recommended to use the drugs reducing the level of CS (for example, aminophenylbutyric acid and magnesium). BJHS does not have an explicit association with CS, but this fact should be proved in a sample with larger number of patients.

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54ASM-0174 | The comorbidity of tension-type headache and alexithymia in adolescents

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Background: Headache prevalence in children and adolescents varies from 23% to 51% depending on the duration of observation. Tension-type headache (TTH) occurs on the average in 25% of the population. The aim of the study was to identify the presence or absence of statistically significant correlation between the high level of alexithymia (AT) and TTH in adolescents.

Materials and Methods: The study group included 52 adolescents (13 male and 39 female) with TTH, aged 13-17 years (average age 14.4 ± 1.4 years). Inclusion criteria: presence of TTH for more than 6 months; absence of preventive treatment; correspondence to the criteria of TTH according to the International Classification of Headache Disorders, 3rd Edition. The control group included 45 people (12 male and 33 female) aged 13-17 years (average age 14.9 ± 1.4 years). The level of AT in adolescents was assessed by the Russian version (Esin, 2019) of the Alexithymia questionnaire for children (Riffe, 2006).

Results: The high level of AT (26.2 ± 1.4 points) was registered in 19 (36.6%) adolescents with TTH, 12 adolescents (23%) were in the border zone (22.5 ± 1.3 points). In control group 5 (11.1%) adolescents had the high level of AT, 17 (37.8%) had borderline values of the scale. Differences in frequency were statistically significant ($P = 0.02$).

Conclusions: The study revealed positive correlation of TTH with the high level of alexithymia. AT is a specific cognitive status which is associated with many psychosomatic illnesses, leads to important clinical consequences, and complicates the treatment, so in neurological practice it is advisable to assess the level of AT in adolescents with pain complaints.

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54ASM-0215 | Non-carcinogenic risk in the multidedient receipt of chemicals for the health of the adolescent and adults population under the conditions of the megapolis

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The aim of the work was a comprehensive assessment of the health risk of adolescents in Kazan under the influence of chemical carcinogens. Working methods in methodological approaches based on health risk assessment. On the territory of Kazan for 2014 - 2019 four zones were identified: 1 - Vakhitovsky district; 2 - Kirovsky district; 3 - Soviet; 4 - Privolzhsky. The exposure scenario was formulated taking into account the complex effects of chemicals on the studied age groups of adolescents and adults in urban areas from different environments (atmospheric air, soil, tap water and food) and for various routes of entry into the body (inhaled, oral and cutaneous). When analyzing the individual carcinogenic risk caused by inhalation of heavy metals (TM) (atmospheric air,

soil, water) in the studied areas, it was found that its values in adolescents are in the range of 9.27E-04 - 2.05E-03 and 6.485 E-04 - 1.13E-03. The carcinogenic risk from exposure to atmospheric air in all areas of the city is within 4.15E-05 and the leading place in its formation is occupied by chromium (3.92E-05) and nickel (1.59E-06). The level for other carcinogens did not exceed 10-6. The greatest risk from inhalation exposure to TM is exposed to the teenage population living in the 3 and 4 zones of the city, where the individual risk of developing cancer is the highest and almost entirely due to the level of chromium (VI) pollution. The total carcinogenic risk, taking into account oral exposure (drinking water, water of reservoirs, soil and food), ranged 1.12E-03 (1 zone) and 9.21E-04 (4th zone) in adolescents and 7.86E-04 (1 zone) and 6.45E-04 (zone 4) in the adult population. The total carcinogenic risk, taking into account the cutaneous exposure in all areas, corresponds to an acceptable level. Its values were lower than the risks received by inhalation and oral route and the highest were observed in zones 2 and 4 for both adolescents and adults, respectively, 8.4E-04 and 5.76E-04 and for adults, respectively, 05.9E-04 and 4.035E-04. So the highest level of total carcinogenic individual risk in the studied territories of the city is determined in adolescents and adults in the 2nd zone (0.00426 and 0.00298). The magnitude of the total carcinogenic risk of TCR in the studied territories with complex multi-media intake of CV in adolescents and adults corresponds to an alarming level of risk (1.0 x 10-3 - 1.1 x 10-4).

54ASM-0235 | Epidemiological peculiarities of the digestive system morbidity in child population of the republic of tatarstan

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Background: The dependence of the frequency of the newly established incidence of digestive diseases on the volume and area of treatment with pesticides and herbicides in the Republic of Tatarstan (RT). In recent years, not only the growth of the digestive system diseases (DSD), but the change of their structure and pathomorphosis: prevalence of upper gastrointestinal tract pathology, levelling of gender differences, and expansion of age limits of formation are observed. This group ranks second among all diseases in children aged 0-14 in Russia and the RT for many years.
Materials and Methods: The analysis included data from the Center for Information and Analysis of the Ministry of Health of the RT, the Ministry of Ecology and Natural Resources of the RT for the period from 2004 to 2018. The non-parametric Spearman rank-order correlation coefficient was determined year to year, and with 1-4 year lag (the time lag) with an indication of the 95% confidence interval (CI).
Results: Pesticide loads ranked first among ecological risk factors of developing DSD in children of RT, whereas chemical pollution of water bodies and emission of hazardous substances into atmospheric air were significantly lower. For the years of 1993–2018, primary morbidity of DSD at the age of 0–14 years increased from 24.3 to 78.8 cases per 1000 children. The leading diseases were gallbladder and bile duct disorders (23.4–25.5%), gastritis and duodenitis (14.1–18.5%), functional gastric disturbances (6.9–9.6%). Relationship between annual incidence of new cases of DSD in children of RT and the pesticide application area for the years 1999–2018 was identified: Pearson correlation coefficient was equal to 0.88 ($P < 0.001$) at 95% of confidence interval/CI (0.64-0.96). The association remained for three years after exposure, being determined as the effect prolongation. The relationship between primary morbidity of DSD and gross pesticide consumption (2.4 D and glyphosate) on the territory of RT remained significant with a 5-year lag after that.
Conclusions: Correlation analysis revealed statistically highly significant correlation of primary morbidity of DSD prevalence with the area and the consumption of pesticides, herbicides consumption volume. It is important that dependence of primary of DSD incidence on the consumption of pesticides, herbicides consumption volume consumption persisted for up to three-five years. The use of pesticides and increase of consumption of glyphosate-containing herbicides at the present stage can contribute to growth of the newly established incidence of digestive diseases in child population, which defines the importance of following the agrotechnological rules.

54ASM-0240 | Necessity of assessing the impact of habitat factors on the health status of the child population

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Background: Majority of researchers qualify congenital abnormalities (CA) of the child population as risk markers and a group of indicator pathology showing high dependence on chemical contamination of environmental compartments and foods. The danger of CA incidence rise is created both due to

impact on the embryo and the fetus, and injury of the parents' germ cells. Social significance of CA lies in the fact that they are on the list of main pathologies forming child disability and mortality.

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 of the RT, FBHI laboratory 'The Center of Hygiene and Epidemiology in the Republic of Tatarstan' for the period from 2010 to 2017. Exposure assessment was made based on the results of analysis of residual concentrations of DDT and HCCH in foods.

Results: Assessment of chronic dietary risk of isolated and complex entry of DDT and HCCH showed that the probability of children's exposure to residual quantities of pesticides, which could result in negative consequences for health, was high in RT (total hazard coefficient (HI $\geq$ 1.0). The major contribution to the value of total non-carcinogenic risk of HCCH and DDT entry is made by milk and dairy products determining up to 47.8% at the Me and the 95-th Percentile levels. The entry of HCCH with cereals and bakery goods - up to 34.2%, rank second. Application of Pearson parametric correlation method revealed statistically significant relationships between primary CA morbidity and indices of pesticide load per hectare of arable land ($R = 0.77$; $P < 0.02$) and the area of arable land tilled with crop protection chemicals (R = 0.73; $P < 0.05$). Significant relationships were identified only at shift of the data on the environmental status for 1—2 years indicating manifestation of delayed effects of exposure.

Conclusions: Exposure to pesticides supplied with food remains a serious public health problem and should be considered as a risk factor for the incidence of VA in children, taking into account the delayed effects. Epidemiological studies at the level of populations in a region are crucial to alert researchers about geographical trends in prevalence of disorders pointing to possible environmental factors. The detection of patterns of morbidity of children population and especially of particular classes of diseases in such territories is only possible under lasting periods of monitoring.

54ASM-0242 | Dysgraphia in children and adolescents with idiopathic generalized epilepsy treated by AEDs

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Background: The effect of antiepileptic drugs (AEDs) on cognitive functions (CF) is more precisely assessed in idiopathic generalized epilepsy (IGE) as this form of epilepsy influences CF minimally. In 2018 the authors worked out Russian-language neuropsychological battery for the assessment of CF (especially – speech) in children and adolescents (C&A) treated by AEDs. The purpose of this study was to evaluate the changes in written speech in C&A with IGE treated by AEDs.

Materials and Methods: 51 C&A (9-17 years old) were examined, 21 (with IGE) formed the study group (SG), 30 (without neurological diseases) were included into control group (CG). The writing samples in SG were evaluated before AEDs treatment, after 3, 6, 9, 12, 18 months, during the same periods in CG (without treatment). Risk ratios and 95% confidence intervals for unfavorable outcome (UO) were calculated with RevMan 5.3 package. UO between SG and CG was compared (results were considered significant when $P < 0.05$).

Results: Comparative analysis of results in SG and CG: before treatment – RR = 1.9; 95% CI [0.47, 7.64], after 3, 6 months – RR = 1.9; 95% CI [0.47, 7.64], after 9 months – RR = 2.5; 95% CI [0.84, 7.47]; after 12 months – RR = 3.21; 95% CI [1.14, 9.07]; after 18 months – RR = 8.18; 95% CI [1.88,35.53]. Statistically significant difference was registered after 12 months of treatment ($P = 0.03$) with the evident increase after 18 months ($P = 0.005$).

Conclusions: AEDs influence negatively the written speech in C&A with IGE: the decline manifests by the appearance of dysgraphic features in writing samples after 12 months; after 18 months these disorders progress significantly. The results show that epileptologists should control not only the side effects of AEDs that influence somatic health, but also the possible deficiency in the development of cognitive functions.

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54ASM-0281 | Environmental factors and the disease incidence of the child population of kazan

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Background: In 2013, the environmental chemical factor was identified as the second after social factors in terms of its contribution to mortality (21%), morbidity (48%) of the population of the Russian Federation and economic losses (32%).
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), We studied the disease incidence in children of 3-6 years old according to main ecology-dependent groups of diseases on the data from children's polyclinics (CP).The study included 4 zones of the city, which had differences in the level of air pollution, soil and drinking water chemicals.
Results: The distribution of primary cases of neoplasms (NP) in the zones is statistically significantly different: zones 1 and 2 ($P = 0.0104$), 1 and 3 ($P = 0.0039$), 2 and 3 ($P = 0.0039$) and 3 and 4 ($P = 0.0065$). Based on the results of the discriminant analysis, confirmation has been obtained that in the 3rd zone, non-random objects are not random. Their variability makes the most significant contribution to the total set of variability of variables (beta regression coefficient is 8.142). The second most important variables for this zone are Diseases of the skin and subcutaneous tissue (atopic and contact dermatitis) (4.983). The frequency of new cases of diseases of the blood and blood-forming organs (BD) among the children's population increased from 0.9 - 2.5 times in certain zones. In recent years, anemia has been 85.4% - 99.0% in the structure of the class of BD in children under 14 years of age in Kazan. High primary incidence of anemia is recorded in zone 2 (95% CI is 11.1-76.2) and zone 3 (95% CI is 56.3-87.6) characterized by a high level of anthropogenic load. Congenital malformations (CM) and developmental abnormalities (DA) belong to the group of indicator pathology reflecting a high dependence on the chemical environmental factor. A decrease in the level of DA by 2.5 times is noted in zone 4, in others this figure increased by 1.3 - 1.8 times.
Conclusions: A high level of primary incidence of NP, CDA and anemia of children was determined in the 2-nd (CP No. 4) and 3-rd (CP No. 6) zones, characterized by a high level of anthropogenic load. Exposure to environmental pollutants in childhood has long-term consequences, which requires further studies to reduce and minimize risk levels taking into account environmental factors.

54ASM-0299 | Experimental hyperhomocysteinemia initiates oxidative stress in the mother-placenta-fetus system

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Background: Maternal hyperhomocysteinemia (HHC) is a known risk factor for severe pregnancy complications such as preeclampsia, intrauterine growth retardation, and neural tube defects increasing risk of abnormal development in the offspring in later life. A significant role in the development of these complications is played by oxidative stress. Here we examined the effects of maternal hyperhomocysteinemia on development of oxidative stress in the mother-placenta-fetus system.
Materials and Methods: HHC was induced in Wistar rats by administration of methionine at a dose of 0.6 mg/kg of body weight in drinking water. The homocysteine (HC) content in blood serum and brain of embryos was examined. We have assessed effect of HHC on the content of malondialdehyde (MDA), oxidative modification of proteins (3-nitrotyrosine, oxyblot) and superoxide dismutase (SOD) activity in placenta, in maternal blood serum and in blood serum and brain of embryos on the 20th day of pregnancy (E20).
Results: We showed that HHC increases the content of MDA and 3-nitrotyrosine in maternal blood serum. It confirms that HHC leads to the development of oxidative stress in the rats during pregnancy. The data obtained indicate that maternal HHC causes oxidative stress in the placenta (an increase of the level of MDA in its fetal part and oxidative modification of proteins in its maternal part). At the same time, superoxide dismutase activity decreased in maternal part of placenta and did not change in fetal part of placenta. It is also shown that elevated level of HC in fetal blood and brain is accompanied by increase of content of MDA. At the same time, 3-nitrotyrosine content and superoxide dismutase activity did

not change in the fetal brains on E20. Thus, oxidative modification of proteins in the fetal brain may be suppressed under the influence of SOD, but the increase in lipid peroxidation products can initiate the processes of oxidative damage of the forming brain, which is consistent with our previous studies of oxidative stress markers in newborn rats undergoing maternal HHC.

Conclusions: The results obtained indicate that maternal HHC causes oxidative stress in the mother-placenta-fetus system, which can lead to disturbances in the offspring development.

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54ASM-0300 | Moderate hyperhomocysteinemia in pregnant rats leads to oxidative stress, neuronal cell death and misformation of cortical cytoarchitecture in the offspring

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Background: Moderate hyperhomocysteinemia (HHC) is a known risk factor for neurodegenerative diseases in humans. During pregnancy, it also leads to various complications in both maternal organism and developing fetus increasing risk of abnormal development of the brain and its functioning in later life. However, little is known about the mechanisms of homocysteine action on the fetal brain development. Hence, in this study we examined the effects of maternal HHC on oxidative stress markers and apoptosis in brain tissue of rat pups.

Materials and Methods: HHC was induced in pregnant female rats by administration of methionine at a dose of 0.6 mg/kg of body weight in drinking water in the period of days 4-21 of pregnancy. The levels of advanced oxidation protein products were determined in pup brains during the first month of postnatal development. For evaluating neuronal cell apoptosis, DNA damage and the levels of caspase-3 protein expression were also analyzed. Additionally we have assessed the efficiency of the antioxidant defense system in the brains of developing pups. To analyze cortical cytoarchitecture an *i.p.* injection of 5′ethynyl-2′deoxyuridine to pregnant rats was used to label the neurons generated in the fetuses on E14, which later differentiate into the pyramidal cortical neurons of the V-VI layers.

Results: Our data demonstrate that there was a significant increase in the levels of advanced oxidation protein products in the brain of pups subjected to prenatal HHC. They also had increased DNA fragmentation and caspase-3 expression in the brain, accompanied by significant reduction in the levels of superoxide dismutase and total antioxidant activity. Structurally, prenatal HHC decreased the total number of labeled cells in the parietal cortex of newborn pups but increased the number of labeled neurons scattered within the superficial cortical layers, suggesting disruption in neuroblast migration during brain development. These morphological changes may be linked to homocysteine-induced oxidative stress in the neurons. Despite the fact that pro-oxidant, antioxidant system balance, and the level of caspase 3 in the brain of 1-month-old rats subjected to prenatal HHC returned to the control values, there was still a reduction in the total number of pyramidal cortical neurons and an increased number of glial cells.

Conclusions: The data obtained suggest that chronic maternal HHC covering the period of generation of cortical neuronal cells affects formation of cortical architecture, which might result in impaired brain functioning in later life. Supported by RFBR №18-015-00099, 20-015-00388 and Russian state budget assignment №AAAA-A19-119021290116-1, AAAA-A18-118012290373-7.

54ASM-0301 | Experimental hyperhomocysteinemia and the risk of nervous system disorders in female rats and offspring

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Background: Homocysteine (HC) is a natural metabolite, which is formed in the organism because of methyl group transfer from S-adenosylmethionine to various molecules (proteins, nucleic acids, biogenic amines etc.). There are

much data, which describe the mechanisms of neurotoxic action of HC, causing the nervous system disorders. HC may interact with NMDA receptors altering glutamatergic transmission, exerts toxic effects on dopaminergic neurons, initiates neuronal apoptosis, and induces oxidative stress, mitochondrial dysfunction and DNA methylation altering gene expression. However, little is known about the effects of HC accumulation in maternal blood (hyperhomocysteinemia, HHC) on subsequent brain development in embryogenesis and in adult life.

Materials and Methods: HHC was induced by *per os* administration to pregnant or intact Wistar rats of 0.15% aqueous L-methionine solution (0.10-0.15 g) per animal using long-term methionine loading.

Results: Our study showed that maternal HC during pregnancy enhanced astroglia and microglia activation, increased expression of interleukin-1β and p38 MAPK phosphorylation in some parts of the offspring brain in early ontogenesis, which can lead to delaying development of nerve tissue and weakening of different brain functions.

We have shown that HHC induced in adult female rats increases the content of biogenic amines and their metabolites in the hypothalamic structures responsible for the gonadotropin-releasing hormone synthesis and secretion (the medial preoptic area and median eminence with the arcuate nuclei), and decreases in the hippocampus, which can lead to weakening of animals cognitive function. Maternal HHC during pregnancy impaired various types of memory in the pubertal female offspring and decreased content of biogenic amines in the hippocampus and hypothalamic structures. The decrease of MAO activity in placenta under the influence of HHC during pregnancy, points to the possibility of disturbance of the placental barrier for maternal biogenic amines, which can also cause disorders of fetal nervous system development. Conclusions

The data presented have shown the different effect of HHC on monoamines content in hypothalamus and similar action on monoamines content in hippocampus of pubertal female rats and pubertal offspring from females treated by methionine during pregnancy. Our results suggest that early impairments of brain maturation, induced by prenatal HHC, might underlie long-term effects. Metabolic changes observed may be due to the disturbances of placental functioning.

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54ASM-0302 | Maternal hyperhomocysteinemia activates apoptosis in the fetal brain

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Background: Maternal hyperhomocysteinemia (HHC) is a risk factor for a number of pregnancy complications such as preeclampsia, placental abruption, intrauterine growth restriction etc. High homocysteine levels at pregnancy can impair the fetal nervous system development. Neurotoxic effects of homocysteine are mediated in particular by apoptosis induction. Thus, in this study, we investigated the effect of maternal HHC on caspase-3 (major effector of neuronal apoptosis) and caspase-8 (predominant initiator in extrinsic apoptosis pathway) activation in the fetal brain and placenta.

Materials and Methods: HHC in pregnant Wistar rats was induced by chronic daily intraoral methionine administration (dissolved in drinking water, 0.6 mg/kg body weight, on days 4-20 of pregnancy). Protein levels of caspase-3 and caspase-8 isoforms in the whole fetal brain, fetal and maternal parts of the placenta were assessed by Western blot on the 20th day of pregnancy. Additionally, caspase-3 activity was determined spectrophotometrically with Ac-DEVD-pNA as a substrate.

Results: HHC increased caspase-3 activity and active caspase-3/procaspase-3 level ratio in the fetal brain. Active caspase-3 was also increased in the maternal part of the placenta. Caspase-8 active isoform levels were not influenced by HHC, but its pro-form was decreased in the fetal brain and increased in the fetal part of the placenta.

Conclusions: Our data suggest that the fetal brain is more sensitive to the maternal HHC pro-apoptotic effect. The lack of caspase-8 activation allowed concluding that apoptosis activation in the fetal brain executed primarily via the intrinsic mitochondrial pathway.

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54ASM-0331 | Endocrine disorders in children and adults caused by pesticides

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Background: Information on endocrine disorders caused by pesticides is of particular interest, although for today epidemiological evidence is limited. Aim is to identify significance and degree of dependence of incidence rates of endocrine diseases (ED) in children and of type 2 diabetes mellitus and obesity in adult population on the parameters of using pesticides at 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 of the RT for the period from 2004 to 2018.
Results: From 1999, the percentage of obese children increased from 8.4% to 18.4%. Only for 10 years, the incidence of new cases of obesity in RT increased from 167.9 to 229.9 per 100 thousand in children under 14 with maximum value of 349.8 cases in 2009. In RT, the percentage of insulin-independent diabetes in DM on the whole varied over a period of 2004-2018 from 90% to 96.7%, which corresponded to global statistics. The range value of primary DM2 incidence in adult population in these years between administrative units of RT increased from 4.6 (2005) to 36.1 (2018); and that of prevalence - from 2.8 (2005) to 3.8 (2018).The highest growth rate was observed as far the frequency of new cases of obesity (260.5%) and the lowest – DM2 (42.1%). The growth of pesticide areas and volumes on simultaneous increase up to 83.9% of glyphosate preparations resulted in a jump of all primary disease incidence of the child population, and to a greater extent, of ED: for the period of 1993–2018 from 4.0 to 56.7 cases per 1000 children. Correlation coefficient of the incidence of new cases of endocrine pathology depending on the area of pesticide application corresponded to 0.73 ($P < 0.02$).
Conclusions: Greater correlation dependence of the incidence of new cases of endocrine diseases in children on pesticide indices indicate higher sensitivity of children, compared with adults, to pesticides on the territories with high indices of pesticide use. Environmental factors are likely to have double impact on the indices of the child's body resistance. At present stage, the use of pesticides and increase of consumption of glyphosate-containing herbicides on cultivation can contribute to growth of prevalence of endocrine diseases in children and diabetes mellitus and obesity in adult population.

54ASM-0349 | Comparative antioxidant activity evaluation of Rosa rugosa, Rosa acicularis and Rosa canina extracts by luminol-enhanced chemiluminescence

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Background: Rosehip - a representative of the *Rosaceae* family, occupies a special place among fruit crops. The rosehips are rich in phenolic compounds and ascorbic acid and are therefore believed to be potential natural antioxidants. Rosehip berries are a source of valuable biologically active substances of various pharmacological effects. This is a high-tech raw material from which a wide range of food raw materials can be obtained, including plant powders and dry extracts, the use of which will expand the assortment, increase nutritional value, and obtain products with high organoleptic properties and an extended shelf life.
Materials and Methods: Rosa rugosa, Rosa acicularis and Rosa canina ethanol (EtOH) and propylene glycol (PG) fruit extracts of were obtained by maceration. Place of harvest - Russia, Republic of Tatarstan.
For quantitative dynamic assessment of plant antioxidant activity (AOA) of EtOH and PG extracts, the chemiluminescence (CL) method was used using luminol as a phosphor, as well as 2,2′-azo-bis (2-amidinopropane) (AAPH) to activate the luminescence. Moreover, the intensity of CL is a measure of radicals number. Trolox and quercetin were used as standard antioxidants. For estimation of CL value of the samples, TAR (total antioxidant reactivity) and TRAP (total reactive antioxidant potential) measurements were calculated. Based on the measurement of the area under the CL curve, there were estimated the relative inhibitory activity of each sample.

Results: Among three studied EtOH extracts of rosehips rosehips, *Rosa canina* possessed the highest rate of interaction of the antioxidant with radicals (the rate of quenching of CL at a concentration of 0.1 mg/mL was 46%, while for the remaining extracts this parameter was 29% or less), as well as the highest TRAP parameter.

Among the extracts obtained by extraction using propylene glycol, the most distinguished was the extract of *Rosa rugosa* hips (on average, TAR values up to a concentration of 0.00001 mg/mL were 43-47% higher than in other studied species and 90-97% higher in terms of TRAP in concentrations up to 0.001 mg/mL).

PG extracts in comparison with EtOH extracts showed an order of magnitude better ability to bind free radicals (0.1 mg/mL for EtOH extract versus 0.0000001 mg/mL for PG) in all studied parameters.

Conclusions: Propylene glycol as a solvent revealed a greater ability to extract biologically active substances, compared with ethanol. CL analysis revealed rosehips as a valuable material with high AOA, which is advisable to recommend for the enrichment of food products with biologically active substances.

54ASM-0358 | Analysis of antioxidant activity of evergreens in the western sector of the forest and forest-steppe zone of Eurasia

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Background: Recent studies show the connection of some human health disorders with the destructive effect of free radicals, therefore, the search for effective antioxidants is a relevant scientific direction. Previous studies in the field of antioxidant activity (AOA) of evergreens have confirmed their increased ability to accumulate in the assimilating organs a wide range of compounds with pronounced AOA.

Maintaining vital functions of evergreens in the cold season when exposed to low temperatures that inhibit photosynthetic processes, involves the inclusion of adaptive mechanisms associated with interconversion and partial expenditure in wintering leaves of organic compounds accumulated during the growing season, which are sources of trophic and energy resources. At the same time, there is no published data on the characteristics of the assimilating organs biochemical composition of evergreens and their correlation with the AOA of the compounds.

Materials and Methods: The water juices of the needles of plants J*uniperus communis L, Cedrus libani, Pinus sibirica, Pinus sylvestris, Picea abies* and the ethanol juice of the needles of *Thuja occidentalis* were examined for AOA. The place of harvest is Russia, Krasnoyarsk region.

Juices are obtained by cold pressing with preliminary grinding of freshly cut needles. The total flavonoid content (TFC) was determined by the photometric method. The ascorbic acid content was determined by titration of the juice with a solution of 2,6-dichlorophenolindophenol. For a quantitative dynamic assessment of AOA of ethanol and propylene glycol extracts, we used the chemiluminescence (CL) method using luminol as a phosphor, as well as AAPH to activate the luminescence.

Results: The most stable total reactive antioxidant potential, i.e. the largest amount of antioxidant was observed in *Juniperus communis L* juice, the longest - in *Thuja occidentalis, Pinus sibirica, Cedrus libani*. These data correlate with the highest TFC, i.e. the longer the latent period, the higher the TFC value in the juice.

According to the parameter of total antioxidant reactivity, *Pinus sylvestris, Juniperus communis L* were characterized by the highest total AOA, while the ascorbic acid content in these juices was the smallest.

Conclusions: The investigated conifer juices slightly differ in the content of antioxidant substances, and however, all juices demonstrate medium strength AOA in dry matter concentrations up to 10^{-3} mg/mL. The manifestation of prooxidant properties may be due to the formation of ascorbic acid conversion products in the CL system. An increase in the TFC may cause an increase in the latent period, or an increase in the total reactive antioxidant potential.

S5 – MICROBIOTA: TOO MANY, TOO GOOD OR TOO BAD

54ASM-0112 | High levels of intestinal-activated IgA+ B lymphocytes support the pathogenic role of intestinal mucosal hyperresponsiveness in IgA nephropathy (IgAN) patients

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Background: IgAN is the most frequent primitive glomerulonephritis. In the last years, the role of mucosal immunity in IgAN has gained importance. Particularly interesting is the role of the microbiota and intestinal immunity in IgAN, due to the activity of secretory IgA in the intestinal mucosa. Here we studied the intestinal-renal axis connections analyzing levels of BAFF, April and intestinal-activated B cells in IgAN patients.
Materials and Methods: Serum and fecal samples were collected from 44 IgAN patients and 23 healthy subjects (HS) with similar clinical features. BAFF and APRIL serum levels were measured by ELISA assay. Metabolomic analysis of the fecal microbiome was performed by mass spectrometry. B cell subsets were identified by FACS.
Results: IgAN patients had increased serum levels of Baff cytokine ($P = 0.012$)correlating to higher amounts of 5 specific microbiota metabolites. Moreover, we found also high April cytokine serum levels in IgAN patients. In addition, we found that subjects with IgAN have a higher proportion of circulating Breg activated at the intestinal level (CCR9+INTB7+) compared to HS (%mean ± SEM= 24.6 ± 5.8 and 8.5 ± 2.9in IgAN and HS; p 0.02).
IgAN patients had high levels of CCR9+/INTB7+ memory B cells (13.4 ± 2.8 and 5.51 ± 1.3 in IgAN and HS; $P = 0.006$) and of intestinal IgA-producing memory B cells (CCR9+/INTB7+/IgA+;22.2 ± 3.5 and 10.3 ± 3.3 in IgAN and HS; $P = 0.03$). Interestingly, they were significantly increased in IgAN patients but not in patients with non-IgA glomerulonephritis.
Finally, we found that IgAN patients had high levels both of total plasmablasts(%4.1 ± 0.9 and 1.2 ± 0.6 in IgAN and HS; $P = 0.001$)and of intestinal-activated plasmablasts (23.6 ± 3.8 and 11.2 ± 2.1 in IgAN and HS; $P = 0.01$).
Conclusions: The results of our study showed for the first time an important difference in the amount of intestinal-activated B lymphocytes among patients with IgAN and HS, confirming the hypothesis of the pathogenic role of intestinal mucosal hyperresponsiveness in the IgAN patients. Therefore, this represents an important area of research for new targeted therapies aiming to stop the evolution towards end-stage renal disease.

54ASM-0342 | Polymicrobial biofilms: in vitro modeling and treatment approaches

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Background: While in the biofilm, bacteria become extremely resistant to antimicrobials, biocides and immune system of the host. In most cases bacteria form polymicrobial communities where the bacterial resistance to a wide range of antibacterial agents significantly increases.
Materials and Methods: Using the drop plate analysis, bacterial viability in mono- and mixed biofilms was assessed in the presence of antimicrobials.
Results: Here we show that antimicrobials being efficient against monoculture become inactive against bacteria embedded into polymicrobial consortium. While under conditions of *S. aureus* biofilm repression by F105 vancomycin, ceftriaxone and tetracycline (inactive against *P. aeruginosa*) lead to complete death of *S. aureus* in monoculture in 24 hours, in a mixed culture *S. aureus* incorporates into *P. aeruginosa* biofilm matrix and survived there. By contrast, ciprofloxacin, amikacin and gentamicin, active against both *S. aureus* and *P. aeruginosa*, reduced the number of CFUs of both *S. aureus* and *P. aeruginosa* in the mixed biofilm by over than 3 orders of magnitude at concentrations 4 and more times lower in compare with monocultures of both bacteria. Moreover, similar increase in antibiotics efficacy was observed when *S. aureus* culture fluid was added to a mature biofilm of *P. aeruginosa*. Even at low concentrations, death of both detached cells and planctonic *P. aeruginosa* cells was observed. This data indicate that *S. aureus* produces certain metabolites at high concentrations leading to the death of *P.aeruginosa*, or increasing the efficiency of antibiotics targeting *P. aeruginosa* even in the biofilm.
Conclusions: While an exact metabolites providing these effects remain to be identified, these findings open promising perspectives to increase the antimicrobial treatment efficacy of the biofilm-associated infections.
This work was supported by the Russian Foundation for Basic Research, grant No 20-04-00247.

54ASM-0377 | Effects of antibiotics and lactobacilli on motor behavior and oxidative stress in mice

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Background: Gut microbiota is a community of bacteria in the intestine that helps to maintain a dynamic and metabolic balance in the body. Changes in microbiota contribute to various diseases including depression, anxiety-phobic states. In our study we analyzed the motor behavior and the level of oxidative stress of mice with altered microbiota.
Materials and Methods: Experiments were performed on mice divided into 3 groups: 1) a control group (i.p. injections of saline, n = 20); 2) mice receiving injections of antibiotics (cocktail of Neomycin, Vancomycin, Amphotericin B, Ampicillin, Metronidazole), AB group, n = 20); 3) mice receiving injections of antibiotics together with supplementation of lactobacilli (4×10^6 cells/mL, AB+LB, n = 20). The motor behavior was assessed in Open Field and Rotarod tests.
Results: In control animals no changes were observed in all tests. AB group demonstrated a significant increase in horizontal activity compared to control and AB+LB groups which may indicate the elevation of the anxiety. Vertical activity in the AB group did not change significantly. The shorter time spent on the rotating cylinder in Rotarod test was shown for AB group ($P < 0.05$) compared to control and AB+LB groups. The level of malone dialdehyde increased in muscle and brain tissues of AB group compared to control and AB+LB groups. The activity of glutathione peroxidases decreased only in muscles. Concentration of glutathione decreased in the AB group and lactobacillus treatment restored its concentration to control values.
Conclusions: Thus, disturbance of the gut microbiota in mice leads to impaired motor coordination and increased anxiety accompanied by higher production of the reactive oxygen species. Supplementation with lactobacilli prevented the observed changes, which indicates on a positive effect of normal microflora on the motor function of mice which is partially mediated by the reduction of the level of oxidative stress. This study was supported by RFBR/Government of Republic of Tatarstan №18-415-160005.

S6 – MEMBRANE TRANSPORTERS AND CHANNELS: TRANSLATING BASIC RESEARCH TO DRUG DISCOVERY AND PRECLINICAL DEVELOPMENT

54ASM-0004 | Cell-attached current-clamp recordings from cortical neurons

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The Arctic zone is a strategically important object for many countries of the world, including the Russian Federation which owns about 6 million km^2, including 2.2 million km^2 of land suitable for life. However, the living conditions in the Arctic radically differ in a number of factors in comparison with other territorial zones of the Russian Federation, which certainly affects the quality of people life in this region. An unbalanced diet in conjunction with a negative impact on the processes of acclimatization and immunity contributes to the prevalence of risk factors for noncommunicable diseases and alimentary-dependent pathologies (arterial hypertension, dyslipoproteinemia and overweight body) among the non-indigenous population of the Arctic zone. According to the results of epidemiological studies conducted in the Yamal-Nenets Autonomous District an average rate of arterial hypertension equal to 31.0% among non-indigenous population was revealed. Thus, the processes of adaptation of non-indigenous populations in the Arctic place high demands on diets. In frame of ITMO University project the norms of consumption of basic nutrients and energy were analyzed according to the recommendations of the Russian Federation and WHO, certain differences were identified and the most adequate macro nutrient requirements for the development of non-indigenous diets in the Arctic zone were determined. In addition, methods were proposed for enriching these diets with essential components of food, in particular minerals and vitamins, in order to prevent a number of noncommunicable diseases.

54ASM-0011 | Constitutive activation of stim1 causes tubular aggregate myopathy: characterization of myoblasts and myotubes deriving from affected patients for the identification of new therapeutic targets.

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Background: Tubular aggregates myopathy (TAM) is a hereditary ultra-rare muscle disorder actually without a cure characterized by progressive weakness, myalgia or myasthenic figures. Biopsies from patients with TAM show the presence of tubular aggregates (TAs) originated from sarcoplasmic reticulum (SR) (Bohm 2018). TAs formation is triggered by functional consequences due to disruption in the SR-T-tubule junction, such as altered Ca2+ homeostasis (Lee 2016). TAM is caused by gain-of-function mutations in STIM1 (Bohm 2013) or ORAI1 (Nesin 2014), proteins responsible for Store-Operated-Calcium-Entry (SOCE), a pivotal mechanism in cellular calcium signalling and in maintaining cellular calcium balance (Cho 2017). However, the mechanisms underlying muscle weakness and TAs formation from altered Ca2+ homeostasis in skeletal muscle of affected individuals remain to be clarified. Thus, this study aims to explore how calcium homeostasis dysregulation could be associated with TAM. Materials and methods: We performed a functional characterization of myoblasts and myotubes deriving from patients carrying Leu96Val STIM1 mutant by using a plethora of techniques raging from fura-2 cytofluorimetric and High Content Imaging technology to real-time PCR. Results: We demonstrated a significantly higher resting calcium concentration and an increased SOCE activity in STIM1 mutants compared with controls. A gene expression analysis highlighted an altered expression of genes coding for proteins regulating calcium handling and myogenesis in STIM1 mutants compared with controls. Finally, a reduced cell multinucleation and mitochondrial vitality was detected in STIM1 mutants indicating an altered fusion process associated to TAM. Conclusions: Our functional study provide a correlation between altered Ca2+ homeostasis and TAM symptoms suggesting that preventing excessive Ca2+ influx and TAs formation could be a therapeutic approach for TAM.

54ASM-0033 | Genistein and 17β estradiol counteract hepatic fatty degeneration by mechanisms involving mitochondria, inflammasome and kinases.

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Background: Oxidative stress and mitochondria dysfunction could be involved in the onset of non-alcoholic fatty liver disease (NAFLD) and in its transition towards non-alcoholic steatohepatitis (NASH). Estrogens/phytoestrogens could counteract liver fat deposition with beneficial effects against NAFLD by unclear mechanisms. We aimed to analyze the protective effects elicited by genistein/estradiol in hepatocytes cultured in NAFLD-like medium by examining cell viability, triglycerides accumulation, mitochondrial function and oxidative stress and role of pathways involving NLRP3 inflammasome, toll like receptors 4 (TLR4), Akt and 5′ AMP-activated protein kinase (AMPK)α1/2
Materials and Methods: Human primary hepatocytes and hepatoma cell line (Huh7.5 cells) were incubated with a 2 mM mixture of two free fatty acids (oleate/palmitate) for 3 hours (NAFLD-like medium) in the presence/absence of tumor necrosis factor (TNF) α. In some experiments, Huh7.5 cells were exposed to genistein and 17βestradiol in the presence/absence of specific inhibitors of Akt, AMPKα1/2, TLR4, NLRP3 inflammasome and estrogenic receptors (ERs). After each treatment, specific assays were performed
Results: Genistein/17βestradiol protected hepatocytes against NAFLD-like medium, by preventing the loss of cell viability and mitochondrial function and the increased ROS release and triglycerides accumulation. Furthermore, both agents counteracted the deleterious effects of TNFα. The blocking of Akt, AMPK and ERs was able to reduce the above effects, which were potentiated by NLRP3 inflammasome.
Conclusions: Our findings suggest novel mechanisms at the basis of protective effects elicited by phytoestrogens and estrogens against NAFLD and NASH and open novel therapeutic perspectives for the management of NAFLD in postmenopausal women.

54ASM-0034 | Exposure to serum from NAFLD patients affects hepatocyte viability, generates mitochondrial dysfunction and modulates pathways involved in fat accumulation and inflammation

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Background: Changes of lipidic storage, oxidative stress and mitochondrial dysfunction may be involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Although our knowledge of intracellular pathways involved in the pathogenesis of NAFLD, the role of circulating triggering factor(s) is debated. We, therefore, aimed to test the hypothesis that factors circulating in the blood of NAFLD patients may influence processes believed to play a role in the pathogenesis of their disease, *in vitro*.

Materials and Methods: Huh7.5 cells were exposed to serum from 12 NAFLD patients and 5 control subjects, in the presence or absence of NLRP3 inflammasome inhibitor, MCC950. Specific assays were performed to verify hepatocyte viability, release of free oxygen species (ROS), hydrogen peroxide, markers of lipidic peroxidation and mitochondrial function. The involvement of NOD-like receptors, such as inflammasome NLRP3, and of signaling related to peroxisome proliferator activating ligand receptor γ (PPAR-γ), sterol regulatory element binding protein 1c (SREBP-1c), nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB), and NADPH oxidase 2 (NOX2) was studied by western blot. Finally, the differential effects of adding tumor necrosis factor (TNF)-α to serum from patients and controls were observed.

Results: Serum of 12 NAFLD patients was able to reduce cell viability and mitochondrial membrane potential by about 48% and 24% ($P < 0.05$) and to increase peroxidation and triglycerides content, in Huh7.5 cells. An increased expression of SREBP-1c, PPARγ, NF-kB and NOX2 of about 51%, 121%, 63% and 46%, respectively, was observed ($P < 0.05$). The above effects were potentiated by pretreatment of Huh7.5 cells with TNF-α and reduced in Huh7.5 cells stimulated with MCC950. No changes in all above parameters were observed with control subjects serum.

Conclusions: In Huh7.5 cells, exposure to serum from NAFLD patients induces a NAFLD-like phenotype by interference with NLRP3 inflammasome pathways and activation of intracellular signaling pathways related to SREBP-1c, PPARγ, NF-kB and NOX2.

54ASM-0041 | Contribution of calcium-activated and voltage-gated potassium channels in pinacidil effects on the isolated internal mammary artery grafts from patients with type-2 diabetes mellitus

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Background: Previously, we have shown that pinacidil, a potassium channel opener, relaxed human internal mammary artery (HIMA) obtained from patients with type-2 diabetes mellitus (T2DM). Additionally, we confirmed that an interaction with smooth muscle ATP-sensitive potassium (K_{ATP}) channels is not involved in the dilatation of HIMA induced by pinacidil. Thus, the aim of our study was to investigate the contribution of calcium-activated (K_{Ca}) and voltage-gated (K_V) potassium channels in pinacidil effects on HIMA obtained from T2DM patients.

Materials and Methods: The segments of HIMA obtained from the diabetic patients undergoing a coronary bypass surgery, without endothelium, were mounted in an organ bath system and an isometric tension was being recorded. The various selective and nonselective blockers were used to identify different potassium channels involved in relaxation effects of pinacidil (0.01 - 100 μM) on HIMA precontracted with 5-hydroxytryptamine (0.1 mM). The experiments followed a multiple curve design.

Results: Tetraethylammonium (1 mM, n = 6), a nonselective blocker of K_{Ca} channels and iberiotoxin, a highly selective blocker of large conductance K_{Ca} (BK_{Ca}) channels (0.1 μM, n = 6) did not antagonize the vasodilatory effects of pinacidil on HIMA ($P > 0.05$). 4-aminopyridin (1 mM and 3 mM, n = 6 both), a nonselective blocker of K_V channels antagonize the vasodilatory effects of pinacidil on HIMA ($P < 0.05$ for both 4-AP concentrations). Application of margatoxin, a highly selective blocker of $K_V1.3$ channels (30 nM, n = 7) did not inhibit relaxation of HIMA produced by pinacidil.

Conclusions: The K_V channels are involved in the endothelium-independent relaxation of diabetic HIMA induced by pinacidil. The $K_{Ca,}$ BK_{Ca} and $K_V1.3$ are not involved in the dilatation of diabetic HIMA produced by pinacidil. It seems, that pinacidil has additional mechanism(s) of action, and that mechanisms on HIMA obtained from T2DM patients include K_V channels different then $K_V1.3$ channel.

54ASM-0062 | Aquaporins-mediated hydrogen peroxide permeability is crucial for human spermatozoa functioning and fertility

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Background: Human spermatozoa express different aquaporins (AQPs) localized both in the plasma membrane (AQP3, AQP7, AQP8) and in intracellular structures (AQP3, AQP11). AQPs have a role in cell volume regulation and cytoplasm removal during sperm maturation. Several studies support that some AQPs act as peroxiporins by facilitating hydrogen peroxide crossing of the cell plasma membranes. Here we aimed to assess the role of peroxiporins in reactive oxygen species (ROS) scavenging in both normospermic and sub-fertile human subjects and the possible effect of human Papillomavirus (HPV) infection.
Materials and Methods: We confirmed the AQPs expression and localization by immunoblotting and immunocytochemistry. The water and H_2O_2 permeability in normospermic and sub-fertile subjects was analyzed by stopped flow light scattering method and by a H_2O_2 fluorescence probe, respectively. Finally, the possible effect of HPV on both expression and function of AQPs in sperm cells of infected patients was studied by ELISA, confocal double staining and co-immunoprecipitation.
Results: The expression and localization of AQP3, 7, 8, 11 proteins was confirmed. Sperm cells showed a water and H_2O_2 permeability which was inhibited by H_2O_2, heat stress and the AQP inhibitor $HgCl_2$ and reversed by reducing agents. Sub-fertile patients showed a reduced AQP functionality. ELISA experiments indicated that HPV infection increased AQPs expression in normospermic patients and decreased it in sub-fertiles. Functional experiments demonstrated that HPV infection heavily decreased the water permeability of sperm cells in normospermic samples. Confocal immunofluorescence showed a colocalization of HPV L1 protein with AQP8, confirmed by co-immunoprecipitation experiments.
Conclusions: Present findings suggest that: 1) AQPs are involved in volume regulation and in ROS elimination, 2) There is a relationship between AQP functioning and both sperm number and motility, 3) HPV infection affects AQPs expression and directly inhibits sperm cells functionality probably by making them more sensitive to oxidative stress.

54ASM-0081 | Preclinical studies neurotropic effects of cobalt acetylsalicylate on neurons Helix albescens Rossm

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Background: The aim of this work is to conduct preclinical studies of aspirin derivative neurotropic effects - cobalt acetylsalicylate (ASC) - on the Helix albescens mollusk's neurons.
Materials and Methods: The experiments were performed on 32 Ppa1 neurons of ganglion complex, which was constantly washed by Ringer's solution (composition in mmol/l: sodium chloride-100, potassium chloride-4, calcium chloride-10, magnesium chloride-4, Tris-HCl-10, pH 7.5). The electrical potentials of neurons were registered (18-21 °C) using the intracellular lead method (amplifier Axoclamp 900 (USA), microscope OLYMPUS BX51 (Japan) and signal digitization Digidata 1550B (USA). Recording potentials and calculating their characteristics in the pClamp 10 software package. Statistical data processing was performed using the nonparametric Wilcoxon criterion.
Results: In Ppa1 neurons, the ASC significantly increased the pulse repetition rate - at a concentration of 5 μmol/l by 38.8% ($n = 10$, $P \leq 0.01$), 50 μmol/l – by 57.1% ($n = 11$, $P \leq 0.01$), and 50 μmol/l – by 56.3% ($n = 11$, $P \leq 0.01$). The ASC at concentrations of 5 and 50 μmol/l significantly increased the maximum increase in total incoming transmembrane ion currents by 56 ($n = 10$, $P \leq 0.05$) and 30.2% ($n = 11$, $P \leq 0.05$), respectively, and at concentrations of 500 μmol/l it significantly reduced them by 22% ($n = 11$, $P \leq 0.05$). The rates of total outgoing transmembrane ion currents decreased by 26.4% at a concentration of 5 μmol/l ($n = 10$, $P \leq 0.05$), while at concentrations of 50 and 500 μmol/l they increased by 37.3% ($n = 11$, $P \leq 0.05$) and 33.3% ($n = 11$, $P \leq 0.05$).
Conclusions: ASC has an activating neurotropic effect (5-500 μmol/l), which is associated with an increase (5-50 μmol/l) or decreased (500 μmol/l) permeability of plasma membranes of neurons are sodium ions, and a reducing (5 μmol/l) or increasing (50-500 μmol/l) - for potassium ions. ASC can be recommended for testing as a tool that has a stimulating effect on the nervous system.
The reported study was funded by RFBR, project number 20-33-70142.

54ASM-0119 | Lack of Aquaporin-9 impairs the systemic inflammatory response 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 following bacterial infection, leading to multiple organ failure and dramatically high mortality (42% at 28 days after diagnosis). Aquaporin-9 (AQP9), a membrane channel protein expressed mainly in hepatocytes and leukocytes, has been recently associated with inflammatory and infectious responses, thus triggering strong interest in AQP9 as a potential target for reducing septic shock-dependent mortality. Following our previous *in vitro* work demonstrating that LPS-induced maturation of murine bone marrow dendritic cells depends on AQP9, here we evaluated whether AQP9 is involved in murine systemic inflammation during endotoxic shock.

Materials and Methods: Wild type (*Aqp9*$^{+/+}$; WT) and *Aqp9* gene knockout (*Aqp9*$^{-/-}$; KO) male mice aged 9-12 weeks were submitted to endotoxic shock by i.p. LPS (40 mg/kg) injection and the related survival times were followed during 72 hours. Electronic paramagnetic resonance and confocal microscopy were employed to analyse the nitric oxide (NO) and superoxide anion (O_2^-) production, and the expression of iNOS, COX-2 and NFκB p65, respectively, in the liver, kidney, aorta and heart of animal specimens.

Results: LPS-treated KO mice survived significantly longer than corresponding WT mice, and 25% of the KO mice recovered fully from the endotoxin treatment. The LPS-injected KO mice showed lower inflammatory NO and O_2^- productions and reduced iNOS and COX-2 levels through impaired NFκB p65 expression/activation in liver, kidney, aorta and heart compared to the LPS-treated WT mice. Consistent with these results, treatment of a rodent hepatoma cell line with 25 μM HTS23168, an AQP9 blocker, prevented the LPS-induced increase of inflammatory NO and O_2^-.

Conclusions: Overall, these results suggest involvement of AQP9 in the LPS-induced endotoxic shock and lead to the appealing idea that modulation of AQP9 expression/activity may be a strategy to counteract the systemic inflammation accompanying the septic shock.

54ASM-0128 | Two distinct pathways for processing hippocampal network activity in the receiver network of the medial entorhinal cortex layer V

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Background: The entorhinal cortex (EC) constitutes a major interface between the hippocampus and various regions of the neocortex.

Materials and Methods: We used simultaneous recordings of sharp-wave-ripples (SPW-R) and postsynaptic responses from LV neurons, electrical and optogenetic stimulation.

Results: Multimodal sensory information enters the hippocampal formation via neurons located in superficial layers of the EC. The deeply located layer V receives a substantial part of the hippocampal output. Layer V of the EC can be subdivided into two separate sublayers with different circuit integration. Layer Va (LVa) contains horizontal neurons which form the major source of intra-telencephalic projections. Pyramidal-like neurons in layer Vb (LVb) are the main targets of afferent fibers from CA1 and the subiculum. Their axons reach mostly local targets like hippocampal-projecting neurons of LII and LIII as well as neurons in LVa. This poses the question how information is transferred from hippocampal networks via EC towards the neocortex. We, therefore, investigated the activation of neurons in layer Va and Vb by natural patterns of hippocampal activity, using an in vitro model of SPW-R events. We show that both, LVb and LVa neurons receive direct excitatory input during SPW-R. At the same time, both layers are poorly connected with each other, suggesting that hippocampal output signals are split into two parallel, separate streams of activity. Hippocampal SPW-R do also strongly excite fast-spiking inhibitory interneurons in layer V. Comparisons between both layers reveal a stronger net excitation of LVa neurons, suggesting preferential processing of SPW-R in the output stream towards the neocortex.

Conclusions: In conclusion, we describe that hippocampal activity patterns recruit two largely separate neuronal systems within EC serving.

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54ASM-0138 | The neuroprotective effect of local hypothermia in the rat model of the endothelin-1-induced focal cerebral ischemia

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Background: Hypothermia greatly reduces sensitivity of neurons to hypoxia in various models. Here, we explored neuroprotective effects of local hypothermia in a model of focal ischemia induced in somatosensory rat barrel cortex by 3 hours long epipial application of endothelin-1 (ET-1).This model recapitulates ischemia developing in patients with subarachnoid hemorrhage.

Materials and Methods: The temperature of cortical surface at the site of ET-1 application was maintained by Pelletier element. Hypoxia-induced impairment was assessed through 16-channel recordings of spontaneous and sensory-evoked activity in different layers of cortical column and post hoc histological reconstructions of the ischemic focus from serial brain sections.

Results: Under normothermic conditions (39°C) ET-1 evoked suppression of activity in all cortical layers, which was most severe in superficial layers 2/3, and induced formation of a conic-shape ischemic focus. Local hypothermia (28°C) was induced at the cortical surface at 0, 10 and 60 minutes time lags after ET-1 application. Hypothermia-induced alleviation of functional impairments was observed in all cortical layers, and it was most pronounced in L4 and L5/6 for the hypothermia started 0 and 10 minutes after ET-1 application. However, hypothermia started 60 minutes after ET-1 application exerted little alleviation of functional injury. In agreement with these electrophysiological observations, hypothermia started 0 and 10 minutes after ET-1 application caused nearly 10-fold reduction in ischemic focus size compared to the normothermic condition, whereas hypothermia started 60 minutes after ET-1 application was much less efficient in reducing the size of ischemic focus.

Conclusions: Thus, local hypothermia started shortly after the onset of ischemia exerts pronounced neuroprotective effects in the ET-1 model of focal cerebral ischemia. Because the ischemia onset can be efficiently detected in patients with subarachnoid hemorrhage we propose that local hypothermia could be considered as a method for alleviation of ischemic complication in these patients.

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54ASM-0139 | Negative ultraslow potentials during endothelin-evoked focal ischemia in the rat cerebral cortex

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Background: Negative ultraslow electrical potentials (NUPs) attaining giant values of up to −150 mV were recently discovered in cerebral cortex of patients during focal and global ischemia, and their occurrence and amplitude highly correlated with the level of ischemic injury.

Materials and Methods: Here, we explored ultraslow potentials using 16-channel direct-coupled recordings of the intracortical potential with silicone probes at different cortical depth in the rat barrel cortex during transient ischemia evoked by epipial application of endothelin-1 which lasted for one hour and was followed by two hours long washout of endothelin.

Results: We found that NUPs started developing shortly (~10 min) after the onset of endothelin application and they were heralded by the first wave of spreading depolarization. NUPs then progressively developed through the entire time course of endothelin application attaining maximal values at the end of endothelin application, and they ceased during endothelin washout. NUPs maintained negativity through all cortical layers, but their amplitude was the most ample in deep cortical layers where they attained values of -66 ± 10 mV ($n = 18$ animals) and they were smaller in amplitude at the cortical surface. Level of irreversible functional impairment assessed by measurement of frequency of multiple unit activity after washout of endothelin positively correlated with the NUPs amplitude. Accordingly, post hoc reconstruction of the ischemic lesion from serial brain sections revealed high correlation between the NUPs' amplitude and the volume of the ischemic lesion ($R = 0.68$; $P = 0.003$).

Conclusions: Thus, the main NUPs feature are reproduced during focal cerebral ischemia induced by endothelin in the rat cortex, and this model can be valuable for future investigations of the underlying mechanisms.

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54ASM-0144 | Ca2+-dependent high-conductance channel activity of F-ATP synthase matches the mitochondrial permeability transition pore

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Background: Permeability transition (PT) in mitochondria leads to increased permeability to ions and solutes of the inner mitochondrial membrane (IMM), cessation of ATP synthesis and finally to cell death. PT is mediated by the permeability transition pore (PTP), a Ca^{2+}-regulated channel allowing the passage of ions and molecules up to 1500 Da. PTP, also known as mitochondrial megachannel (MMC), was identified as a high conductance channel by means of patch clamp in mitoplasts in the early '90s. Despite many years of research and the key role of PT in several diseases, including cardiovascular diseases and muscle dystrophies, PTP molecular identity remains a matter of debate and controversy. In 2013 Giorgio et al. provided evidence that dimers of mammalian F_OF_1 ATP synthase purified from native gels can form the PTP/MMC but the exact molecular mechanism is still unclear.

Materials and Methods: F-ATP synthase was purified from bovine heart using the very mild, lipid-like detergent LMNG, and analyzed the preparation with clear native PAGE, SDS-PAGE, mass spectrometry, and negative stain electron microscopy. Protein was incorporated into planar lipid bilayers (PLB), by means of direct reconstitution or proteoliposomes fusion, and channel activity was assessed in the presence of Ca^{2+} and specific PTP activators.

Results: After insertion of fully active F-ATP synthase in preformed liposomes we showed that Ca^{2+} dissipates the H^+ gradient generated by ATP hydrolysis. After incorporation of the same preparation into PLB, channel activity was assessed in the presence of Ca^{2+} and benzodiazepine 423, a specific activator of the MMC/PTP. Currents were inhibited by the typical PTP/MMC inhibitors, such as Mg^{2+} and adenine nucleotides.

Conclusions: Here we provide evidence of high conductance, Ca^{2+}-dependent, channel activity of mammalian F_OF_1 ATP synthase resembling the MMC/PTP key features, including inhibition by PTP specific compounds.

54ASM-0155 | 4-AP model of evoked epileptic activity in the developing sensory cortex in a newborn rat

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Background: Understanding the etiology of neonatal epileptic activity is vital. Only some of the existing models of evoked epileptic activity are effective in the early neonatal states. One of them is the 4-aminopyridine (4-AP) model. However, the lack of consistency in the expected epileptic activity using 4 AP-induced model of epilepsy in the immature nervous system in vivo may give rise to differences in the results and interpretation thereof.

Materials and Methods: Here we decided to answer this question using newborn Wistar rats at the early stages of their postnatal development (P7-11). The epileptic activity was evoked by a local injection of a mixture of epileptogenic drugs 4-AP and bicuculline (100 mM and 1 mM). In order to characterize epileptic activity, the electrical activity of neurons was recorded using a 16 channels silicon probe placed to the epileptic focus detected using intrinsic optical signal imaging.

Results: After a single injection, multiple repeated episodes of evoked epileptiform activity were observed (up to 17 episodes, the average number of episodes was 8.4 ± 0.96, with a frequency of 4.09 ± 0.46 episodes/hour). Spectral analysis of evoked epileptic activity showed a progressive increase in the power of ictal discharges (for the first episode was $2.59 \pm 0.58*10^3$ mV^2/Hz, and for the last episode was $13.9 \pm 8.3*10^3$ mV^2/Hz) without significant changes of the population discharges frequency. There was also an increase in the amplitude of population spikes during the experiment (from -0.24 ± 0.03 mV during the first episode to -0.4 ± 0.07 mV during the tenth episode).

Conclusions: Although our study is still ongoing, based on our results, we can suggest that the 4-AP model of evoked epileptic activity could be an effective model for studying chronic neonatal epilepsy in the developing CNS in vivo.

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54ASM-0156 | Developmental changes of the early sharp waves in the developing hippocampus of the neonatal rat

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Background: One of the earliest neuronal activity patterns in the developing hippocampus is the early sharp waves (eSPW). They are believed to be involved in cognitive processes (memory and emotions). However, despite the important functional role of eSPW in the development of the central nervous system, little is known about their developmental changes during the early postnatal period.
Materials and Methods: To characterize the changes in the amplitude-time parameters of the eSPW, we have done a series of experiments on newborn Wistar rats (P4-P11). Electrical activity was recorded in the CA1 hippocampal region using a 16-channel electrode.
Results: While the most powerful eSPW were observed in the lacunosum-moleculare layer of CA1 (-130.3 ± 25.7 mV), their reversion was seen between the radial and pyramidal layer. eSPWs were characterized by a positively directed LFP deflection (72.48 ± 18.5 mV) in the oriens layer. We also found a developmental increase in the eSPW amplitude in the radial layer from 72.5 ± 16.2 mV (P4) to 105.7 ± 0.5 mV (P11), while in the oriens layer, the eSPW amplitude was weakly changed. There was also a significant decrease in the half-width of the eSPW during the development of the neonatal rat (53.4 ± 4 ms in P4 rats and 30.8 ± 2.5 ms in P11). We have also observed a significant developmental increase of eSPW occurrence (1.8 ± 1.1 eSPW/min on P4, 13.6 ± 0.02 eSPW/min on P11).
Conclusions: In addition to the involvement of eSPW in hippocampal networks formation, eSPW undergos the developmental changes. We also suggest that the amplitude-time parameters of eSPW can serve as a marker of the maturation of the neuronal networks in the developing hippocampus.
The work was supported by RSF grant #16-15-10174 (data analysis) and the subsidy allocated to KFU for the state assignment in the sphere of scientific activities (electrophysiological experiments).

54ASM-0157 | Differential effects of Urethane and Isoflurane on the evoked intrinsic optical signal in the somatosensory system of the neonatal rat

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Background: Biomedical studies are most often performed under anesthesia conditions that significantly improve the quality of the experiment. However, the results of the experiment may be affected by interactions with the anesthetic agent. While the effects of anesthetics in adult animals are the subject of deep research, little is known about the mechanisms of the widely used anesthetic agents on the developing central nervous system. In the present study, we aimed to answer this question by studying the effect of different concentrations of anesthetics (urethane and isoflurane) on induced neuronal activity and local changes in the blood supply in the somatosensory cortex of neonatal rats.
Materials and Methods: To characterize the effect of the anesthetics we used the method of the intrinsic optical signal (IOS) in neonatal Wistar rats (P6-P8). Using different light wavelengths we were able to characterize the tissue component of the IOS that highly correlated with the neuronal activity and hemovascular component of the IOS reflected changes in the blood flow.
Results: The results of our experiments showed the progressive decrease in the tissue component of IOS reflecting attenuation of the neuronal activity due to the increase of the anaesthetics concentration. However, used anesthetics differently affected the hemovascular component of the IOS. While at low concentrations of anesthetics the hemovascular response was highly modulated by urethane, use of the high dosage of anesthesia showed that isoflurane strongly affects the level of blood flow.
Conclusions: Altogether, our results demonstrate different effects of the urethane and isoflurane on the blood flow depending on the used concentration. We suggest that in the studies on the developing central nervous system this phenomena should be taken into account. The work was supported by RSF grant 16-15-10174.

54ASM-0158 | Detection of focal epileptic activity in the developing somatosensory cortex of newborn rat using the intrinsic optical signal technique

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Background: The early stages of postnatal development are characterized by a high level of excitability in the developing brain, resulting in an increased risk of epileptic activity emergence. Thus the diagnosis is a vital task to prevent negative consequences in the future. Earlier, we have already demonstrated the high efficiency of the intrinsic optical signal imaging (IOS) in detecting the position of evoked neuronal activity in the developing somatosensory cortex. We hypothesized that the IOS technique could also be effective for detecting the focus of the epileptic activity and for describing its spatial propagation.
Materials and Methods: To test our hypothesis, we did a series of experiments on neonatal rats of age p7-11, where epileptic activity was evoked by local injection of 4-aminopyridine (100 mM) and bicuculline (1 mM). In order to determine the temporal features of epileptic activity, the electroencephalographic recording was also done using a multi-channel electrode.
Results: The results of our experiments showed that a single injection evoked multiple, repeated episodes of epileptic activity (6.7 ± 0.8 episodes within 1.5 hours after injection). Video analysis showed that in 96.1% of cases, electrographic epileptic activity was accompanied by IOS. The average duration of epileptic IOS was 334.8 ± 42.8 sec, which was slightly different from the duration of epileptic activity recorded electrographically (326.2 ± 20.9 sec). An analysis of IOS also showed that the site of the generation was close to the site of injection.
Conclusions: Based on our results, we suggest that the IOS technique is highly efficient in detecting the focus of epileptic activity and in describing its spatio-temporal characteristics.
The research was supported by RSF (16-15-10174).

54ASM-0159 | Multispectral optical intrinsic signal imaging in the neonatal rat cortex

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Background: Optical intrinsic signal (OIS) is a perspective and minimally invasive method of the visualization of the neonatal neuronal activity. It was shown that dominant mechanisms underlying the OIS are local changes in the blood flow, blood oxygenation and light scattering of the neuronal tissue. While the light parameters to characterize the mechanisms underlying OIS were established in adult animals, little is known about the features of OIS registration necessary to decipher these mechanisms in the developing nervous system
Materials and Methods: We have attempted to answer this question using modified Beer–Lambert–Bouguer law with light scattering correction and developed a method for estimation changes in the concentration of the oxy-, deoxyhemoglobin and tissue light scattering in the neonatal rat cortex. The experiments were done using the multispectral recordings of the sensory-evoked OIS in the somatosensory cortex in the neonatal rats (P6-7 days old).
Results: Based on our results, we showed that the set of 3 any wavelengths of the visible part of the electromagnetic spectrum could be used to characterize the underlying OIS mechanisms. The significant restriction was only imposed by the light absorption by the tissue on the selected wavelength. Independence on the light wavelengths allowed us to increase the depth of light penetration by shifting the wavelength range to the red and near-infrared part of the visible spectrum.
Conclusions: Thus, despite the low amplitude of the evoked OIS in the immature neocortex (<1%), OIS imaging could be perfectly used to investigate the changes in hemovascular and tissue components associated with the neuronal activity in the immature central nervous system. We also suggest that modified OIS technique could be implemented in human brain research. This work was supported by RSF grant 16-15-10174.

54ASM-0381 | Electrophysiological and behavioral study of flurothyl induced seizure in rats with prenatal hyperhomocysteinemia

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Background: Homocysteine (Hcy) is a non-protein sulfur-containing amino acid formed during intracellular conversion of methionine to cysteine. Increased blood levels of Hcy, known as hyperhomocysteinemia (HHcy) displays various neurological abnormalities, such as mental retardation, seizures, and Alzheimer's disease. Increased Hcy level may attenuate the function of GABA(A) receptor, leading to disruption in blood brain barrier. However, susceptibility to seizures in rats with prenatal HHcy in vivo was not investigated.
Materials and Methods: Wistar rats of both sexes from second postnatal week were used throughout the study. Extracellular neuronal activity was recorded from hippocampus using 16-site linear silicon probes (Neuronexus technologies, MI) in vivo. Multiunit activity and high-amplitude spike-wave complexes (SWC) were detected and analyzed using MATLAB environment. Seizures were induced by inhalation of flurothyl (0.1 mL) a rapid acting volatile convulsant added to a mask-chamber. In behavioral study limb and body movements were detected by camera and the flurothyl-induced behavioral activities were graded according to a modified Racine's scale :1.initial agitation and increased exploratory activity, 2. head nodding, forelimb clonus, 3. rearing, wild running and jumping, 4. rearing and falling; swimming movements followed by tonus, 5 tonic-clonic seizures.
Results: The background neuronal activity was higher in HHcy animals compared to control group. Administration of flurothyl induced multiunit activity burst and SWC in hippocampus. The amplitude of SWC was higher in HHcy group compared to control. In behavioral study animals from the HHcy group demonstrated shorter latency of flurotyl-induced specific behavioral patterns, according to Racine's scale. The onset latency of tonic-clonic seizures was shorter in rats from the HHcy group and more animals demonstrated the development of tonic-clonic seizures. Tonic-clonic seizures were observed in 66% of control animals and 100% of HHcy animals.
Conclusions: Our results provide experimental evidence that prenatal HHCY increases brain excitability which may underlie higher a risk of epilepsy development in early postnatal life. This work was supported by RFBR № 18-015-00423 and by the subsidy allocated to Kazan Federal University for the state assignment № 0671-2020-0059 in the sphere of scientific activities

54ASM-0389 | Bioscreening zinc acetylsalicylate neurotropic effects on neurons Helix albescens Rossm

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Background: The aim of the work is to conduct preclinical bioscreening of aspirin derivative neurotropic effects - zinc acetylsalicylate (ASZ) - on the *Helix albescens Rossm.* mollusk's neurons.
Materials and Methods: The experiments were performed on 33 Ppa1 neurons of ganglion complex, which was constantly washed by Ringer's solution (composition in mmol/l: sodium chloride – 100, potassium chloride – 4, calcium chloride – 10, magnesium chloride – 4, Tris-HCl – 10, pH 7.5). The electrical potentials of neurons were registered (18-21 °C) using the intracellular lead method (amplifier Axoclamp 900 (USA), microscope OLYMPUS BX51 (Japan) and signal digitization Digidata 1550B (USA). Potentials were recorded and their characteristics calculated in the pClamp 10 software package in the background and during 5 minutes of ACZ exposure in concentrations 5, 50 and 500 μmol/l. Statistical data processing was performed using the nonparametric Wilcoxon criterion.
Results: In Ppa1 neurons, the ASZ significantly increased the pulse repetition rate compared to the background – at a concentration of 5 μmol/l by 111.1% ($n = 11$, $P \leq 0.01$) and at a concentration 500 μmol/l – by 33.3% ($n = 11$, $P \leq 0.01$). The ASZ at concentrations of 5 and 50 μmol/l significantly increased the maximum increase in total incoming transmembrane ion currents compared to the background by 68.4 ($n = 11$, $P \leq 0.01$) and 40.8% ($n = 11$, $P \leq 0.05$), respectively, and at concentrations of 500 μmol/l it significantly reduced them by 30.5% ($n = 11$, $P \leq 0.05$). The rates of total outgoing transmembrane ion currents increased compared to the background by 38.0% at a concentration of 50 μmol/l ($n = 11$, $P \leq 0.05$) and at a concentration 500 μmol/l – by 29.6% ($n = 11$, $P \leq 0.05$).
Conclusions: ASZ has an activating neurotropic effect (5 and 500 μmol/l), which is associated with an increase (5 μmol/l) or decreased (500 μmol/l) permeability of plasma membranes of neurons are sodium ions, and increasing (500 μmol/l) - for potassium ions. ASZ can be recommended for testing as a tool that has a stimulating effect on the nervous system.
The reported study was funded by RFBR, project number 20-33-70142.

54ASM-0318 | Towards a personalized medicine for Bartter's syndrome: functional characterization of novel CLCNKB mutations and chaperone efficacy of ClC-Kb ligands

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Background: Type III and IV Bartter syndromes (BS) are rare kidney tubulopathies caused by loss-of-function mutations in the *CLCNKB* and *BSND* genes coding for ClC-Kb chloride channels and accessory subunit barttin. ClC-K channels are expressed in the Henle's loop, distal convoluted tubule and cortical collecting ducts of the kidney and contribute to chloride absorption and urine concentration. We identified two new mutations in *CLCNKB,* G167V and G289R, in children affected by BS and a previously reported mutation, A242E. This latter mutation reduced ClC-Kb membrane expression and chloride current levels. All the patients had hypokalemia and metabolic alkalosis, increased serum renin and aldosterone levels and were treated with a symptomatic therapy. The aim of this study was to characterize the new ClC-Kb BS mutations, G167V and G289R. In addition, we tested the hypothesis that ClC-Kb ligands, niflumic acid, valsartan and SRA-36, can act as pharmacological chaperones of ClC-Kb mutants G167V and A242E.
Materials and Methods: We co-expressed ClC-Kb WT and mutant channels with barttin in HEK293 cells and recorded chloride currents through the patch-clamp technique. For the pharmacological experiments, transfected cells were incubated for 24 hours with ClC-Kb-targeting drugs and chloride currents were recorded after drug washout. Confocal microscopy was used to detect membrane expression for ClC-Kb WT and mutants.
Results: G167V channels showed a drastic current reduction compared to WT, likely suggesting compromised expression of mutant channels on the plasma membrane and confirming a genotype-phenotype correlation. The same defects were confirmed for the A242E channels. Conversely, G289R channel was similar to WT raising the doubt that an additional mutation in another gene or other mechanisms could account for the clinical phenotype. Interestingly, increasing ClC-K/barttin ratio augmented G167V and A242E mutants chloride current amplitudes towards WT levels. In addition, niflumic acid 100 mM, valsartan 50 mM and the benzofuran derivative SRA-36 5 mM increased G167V and A242E chloride currents amplitudes towards WT levels. As expected, neither losartan 50 mM nor carbamazepine 100 mM, two drugs that show poor selectivity against ClC-Kb channels, were able to increase the current amplitude of G167V and A242E channels after 24 hours incubation.
Conclusions: These results suggest that the functional characterization of mutant channels is fundamental both for the genotype-phenotype correlation and to choose the best therapeutic strategy. In this frame, we provide a preclinical proof-of-concept that small ClC-Kb reversible ligands can recover the activity of expression-defective mutants associated with BS, opening the way for a personalized mechanism-based therapy for BS patients.

S7 – MOLECULAR DRIVERS OF NOVEL TREATMENTS: FROM BENCH TO BEDSIDE

54ASM-0004 | Effect of hyperinsulinemia on oxidative modification of proteins in the muscle tissue of laboratory rats

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Background: The aim of this work is to study the effect of hyperinsulinemia on the content of products of oxidative modification of proteins (OMP) in the muscle tissue (MT) of laboratory rats.
Materials and Methods: The material for the experiment was a homogenate of MT of 32 laboratory male rats of the Wistar line (220-240 g). Laboratory rats were divided into 4 groups of 8 animals. The intact group was administered saline solution, group I — insulin 1 time per day; group II — insulin 1 time per day for 2 days; group III — insulin 1 time per day for 3 days. Insulin was administered subcutaneously in an amount of 3.5 units. The presence of hypoglycemic coma was determined by the appearance of seizures. In all groups, the level of OMP products was determined at wavelengths of 356, 370, 430 and 530 nm by a method based on the registration of 2,4-dinitrophenylhydrazine derivatives.

Results: It was found that hyperinsulinemia leads to a significant decrease in the MT of laboratory rats in comparison with the intact group of the content of OMP products: in group I — by 13.3% ($P \leq 0.05$) at 356 nm; in group II — by 20% ($P \leq 0.05$) at 356 nm, 30.8% ($P \leq 0.001$) at 370 nm, 27.3% ($P \leq 0.01$) at 430 nm, 21.2% ($P \leq 0.01$) at 530 nm; in group III — by 26.7% ($P \leq 0.01$) at 356 nm, 33.1% ($P \leq 0.01$) at 370 nm, 19.2% ($P \leq 0.05$) at 430 nm, 33.3% ($P \leq 0.001$) at 530 nm. This indicates a slight decrease in free radical oxidation processes in 1-day hyperinsulinemia and a more significant decrease in 2-and 3-day hyperinsulinemia.
Conclusions: The results of the study showed the protective role of hyperinsulinemia in MT when modelling oxidative stress and are consistent with previously obtained data under similar conditions for nervous tissue. This explains why hyperinsulinemia often accompanies cardiovascular pathologies.

54ASM-0005 | Effect of hyperinsulinemia on oxidative modification of proteins in laboratory rat liver homogenate

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Background: The aim of this work is to study the effect of hyperinsulinemia on the content of products of oxidative modification of proteins (OMP) in the liver homogenate of laboratory rats.
Materials and Methods: The material for the experiment was the liver homogenate of 32 Wistar male laboratory rats (220-240 g). Laboratory rats were divided into 4 groups of 8 animals. The intact group was administered saline solution, group I — insulin 1 time per day; group II — insulin 1 time per day for 2 days; group III — insulin 1 time per day for 3 days. Insulin was administered subcutaneously in an amount of 3.5 units. The presence of hypoglycemic coma was determined by the appearance of seizures. In all groups, the level of OMP products was determined at wavelengths of 356, 370, 430 and 530 nm by a method based on the formation derivatives of 2,4-dinitrophenylhydrazine.
Results: It was found that hyperinsulinemia leads to a significant decrease in the liver homogenate of laboratory rats in comparison with the intact group of the content of OMP products: in group I ($P \leq 0.001$) — by 46.2% at 356 nm, 83.3% at 370 nm, 72.7% at 430 nm, 71.4% at 530 nm; in group II ($P \leq 0.05$) — by 15.4% at 356 nm, 16.7% at 370 nm, 18.2% at 430 nm, 28.6% at 530 nm; in group III ($P \leq 0.01$) — by 30.8 at 356 nm, 33.3% at 370 nm, 36.4% at 430 nm, 42.9% at 530 nm. These results indicate a marked decrease in the processes of free radical protein oxidation in 1-, 2-, and 3-day hyperinsulinemia.
Conclusions: A significant decrease in the processes of free radical oxidation of proteins in hyperinsulinemia indicates the protective role of hyperinsulinemia in liver tissues from damaging factors of oxidative stress. This may be the reason why hyperinsulinemia accompanies many liver pathologies.

54ASM-0018 | IL-8 in circulating neutrophils as a potential biomarker in ovarian cancer

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Background: IL-8 is an inflammatory cytokine involved in the development of a number of hallmarks of cancer. One of the main producers of IL-8 is neutrophils (Nph). The aim of the study was to evaluate the biomarker potential of IL-8 in ovarian cancer (OC).
Materials and Methods: The study included 108 patients with primary epithelial OC I-IV FIGO stage and 54 patients with benign ovarian tumors. The levels of IL-8 were determined by ELISA (JSC 'Vector Best', Russia) in the Nph lysate and blood serum before treatment. Genomic DNA was isolated from EDTA-stabilized blood, and the IL-8-845C/T polymorphism was determined using the PCR-RFLP (CFX96, Bio-Rad, USA). For statistical significance assessment, the Anova criterion ($P \leq 0.05$) was used (Statistica 13).
Results: We found that the level of IL-8 in blood serum of OC patients is lower compared to patients with benign ovarian tumors ($P = 0.043$). Serum IL-8 levels did not correlate with either stage or progression-free interval (PFI). The level of IL-8 in Nph of III–IV FIGO stage OC patients was higher than in I-II stages and benign ovarian tumors ($P = 0.0006$). In addition, in the group of patients with PFI<6 months, the content of IL-8 in Nph was higher than in the group with PFS>6 months ($P = 0.00085$). Genotyping revealed that the -845C allele (845T/C) was associated with decreased IL-8 production by Nph ($P = 0.042$); the -845T* allele was significantly more common in benign tumors carriers (86%) than in OC (59%). Serum IL-8 levels did not correlate with any allele frequency.
Conclusions: A decrease in the level of IL-8 in blood serum and its increase in Nph can serve as a diagnostic marker in OC. An increase in the level of IL-8 in Nph is a potential marker of poor prognosis in OC. 845T/C polymorphism is associated with a change in the content of IL-8 in Nph.

54ASM-0019 | The glutathione system parameters in tumor cells of ascites as a predictor of ovarian cancer chemoresistance.

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Background: The glutathione system provides one of the main mechanisms of resistance to platinum-containing chemotherapy (CT) in ovarian cancer tumor cells (OC). The aim of this work was to evaluate the components of the glutathione system in cells of OC ascites as the possible predictors of chemoresistance.

Materials and Methods: The study included 40 primary patients with the III-IV FIGO stage OC, who were admitted to the Regional Clinical Oncology Center, Ulyanovsk. Prior to treatment, the activity of glutathione reductase (GR), glutathione-S-transferase (GST), glutathione peroxidase (GPO), and reduced glutathione (GSH) (Applichem, USA) was biochemically determined in the ascites cells with a spectrophotometer Genesys 10S UV-Vis (Thermo Scientific, USA). The patients were divided into groups with the disease progression during CT, with progression-free interval (PFI) <6 months and PFI>6 months after last course of first-line CT. For statistical evaluation, the Anova criterion ($P \leq 0.05$), the Spearman criterion, log-rank test (Statistica 13.0) were used.

Results: We found that the activity of GR in ascites cells in the group with progression during CT was higher than in other patients ($P = 0.049$). In patients with distant metastases, the level of GSH in ascites cells was lower than in the absence of metastases ($P = 0.030$). We found a direct average strength correlation between the content of all the studied components in ascites cells. The low level of GSH in OC ascites cells corresponded to a higher overall survival of patients (median 953 days) compared with the group with hyperproduction of GSH (median 287 days) ($P = 0.039$). High GR activity corresponded to a short progression-free interval (median 44 days vs 144 days in the group with low GR activity, $P = 0.058$).

Conclusions: The determination of the activity of GR and GSH in the cell fraction of ascites in advanced OC can be used to detect primary chemoresistance.

54ASM-0294 | E3 ligase Pirh2 positively regulates c-Myc oncogene in lung cancer cells

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Background: The investigation of novel targets for anticancer therapy does not lose relevance especially for lung cancer since it is the most common cause of cancer death in the world.

In our study we focused on one of the main negative regulators of the p53 oncosuppressor protein – E3 ligase Pirh2. Despite the frequent mention of this protein in the context of p53 ubiquitination and degradation, other targets and functions of this ligase are not well investigated. In order to fill this gap we used p53-negative human non-small cell lung cancer cell line H1299 to study p53-independent Pirh2 functions.

Materials and Methods: We used stable cell lines H1299 with overexpression and knock-down of Pirh2 for most of the experiments presented. Cell proliferation and migration rates were determined by real-time cell index measurement, as well as by colony-formation and wound-healing assays. MTT assay was used for cytotoxicity tests. The apoptosis level was determined using flow cytometry of Annexin-V staining.

Pirh2 interactome was investigated by GST pull-down with subsequent identification of bound proteins by mass-spectrometry. The validation of interacting partners was performed by reciprocal GST pull-down and/or co-immunoprecipitation. We also used MG-132 and cycloheximide for protein stability tests. mRNA and protein levels were determined by RT-PCR and wester blot.

Results: First, we found that Pirh2 acts as an oncogene in p53-negative non-small cell lung cancer cells H1299. Thus, Pirh2 overexpression leads to increased proliferation, migration and resistance to genotoxic stress, while Pirh2 knockdown caused the opposite effect. Then, in order to understand the mechanism of p53-independent carcinogenic effect of Pirh2 we determined the levels of anti-apoptotic factors NF-κB and c-Myc in H1299 with different status of Pirh2. As a result we found that Pirh2 upregulates c-Myc expression both in mRNA and protein levels, while RelA/p65 subunit of NF-κB was unchanged. To reveal the mechanism of Pirh2-mediated c-Myc regulation we identified more than 200 previously unknown Pirh2-binding partners. Among them, we were interested in RNA-binding protein HuR which is known to be a negative regulator of c-Myc expression. We demonstrate that

Pirh2 binds to HuR, ubiquitinates and directs it to proteasome degradation, which in turn leads to a change in the expression of HuR-dependent genes.

Conclusions: The result of this work is the discovery of a new Pirh2-dependent mechanism of cancer phenotype formation. We suggest that Pirh2 is a promising target for developing therapy for this type of cancer.

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54ASM-0296 | Methyltransferase Set7/9 as a prognostic marker and therapeutic target in breast cancer

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Background: Today, breast cancer (BC) is the most commonly diagnosed cancer type among female population in the world. Despite the obvious progress in this field, finding effective approaches for the diagnosis and treatment of BC is an urgent problem.

Set7/9 is lysine-specific methyltransferase that is known to methylate both histones (H1 and H3) and non-histone targets, such as p53, E2F1, YAP, estrogen receptor ER, NFκB. This enzyme is directly involved in such cellular processes as the regulation of the cellular response to genotoxic stress, the cell cycle and apoptosis. Since the role of Set7/9 in the development of BC is not clear, we focused on it in this study.

Materials and Methods: For bioinformatic analysis the open database Gene Expression Omnibus (GEO) was used. Set7/9 knockdown in BC cell lines was achieved using lentiviral shRNA transduction. MTT assay was used for cytotoxicity tests. Commercially available Set7/9-specific inhibitor (R)-PFI-2 was purchased from Merc, Germany.

Results: As a result of bioinformatic analysis we identified the negative correlation between Set7/9 expression level in BC tissues and patients' lifespan. Moreover, we found that the positive status of HER2 is associated with the increased expression of Set7/9 methyltransferase in BC cells. HER2-positive form of BC is characterized by an aggressive course, an unfavorable prognosis and an increased resistance to standard chemotherapeutic drugs, which again emphasizes the importance of finding new therapeutic targets for this form of cancer.

In order to identify role of the Set7/9 in HER2-driven signaling we knocked down Set7/9 in human HER2-positive BC cell line. Using the obtained cell model, we showed that when Set7/9 is downregulated in SKBR cells, the level of HER2 downstream kinases AKT and ERK is decreased. We suggest that Set7/9 plays an important role in the activation of HER2-driven pro-survival molecular cascades and we plan to further investigate the exact mechanism of this impact.

We also showed that the knock-down of Set7/9 as well as the use of a specific inhibitor of methyltransferase activity of Set7/9 (R)-PFI-2, increases the sensitivity of BC cells both HER2-positive and HER2-negative to genotoxic anticancer drugs.

Conclusions: To summarize, the study indicates that Set7/9 expression may be considered as a prognostic marker of BC outcome for both HER2-positive and HER2-negative BC forms. In addition, a specific inhibitors of Set7/9 methyltransferase activity can be potentially used as a part of combined BC therapy to increase treatment efficacy.

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54ASM-0297 | Evaluating the levels of cytokines and peripheral nerve disorders in mouse model amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal and rapidly progressive motor neuron devastating disease. ALS is characterized by degeneration and gradual loss of anterior-lateral horn spinal cord motoneurons. Currently, there are no drugs or adequate treatments available to stop or reverse this disease. Besides, there are no reliable biomarkers for disease diagnostics. Adequate diagnosis of ALS and quantitative monitoring of its progression are essential for the realization of clinical trials. Recently, it has been shown that cytokines, chemokines and growth factors play a significant role in the pathogenesis of various neurological diseases, including ALS.

Materials and Methods: Applying method of light and transmission electron microscopy (TEM), we examined the peripheral sciatic nerves of transgenic ALS mice (B6SJL-TG(SOD1-G93A)dl1Gur/J) at the preclinical, clinical and terminal stages of the disease, as well as sciatic nerves of wild-type mice. Concentration cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, Eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1 (MCAF), MIP-1α, MIP-1β, RANTES, TNF-α) in the serum of these animals was evaluated using the Bio-Plex Pro Mouse Cytokine 23-plex Assay (Bio-Rad).

Results: As clinical manifestations developed, a significant decrease in the number of myelin fibers in the sciatic nerve was detected. The processes of demyelination were accompanied with apoptotic and autophagic processes in Schwann cells, a change in the myelin index without changes in the axon ultrastructure at the preclinical and clinical stages, and replacement of the destroyed nerve elements with fibrous tissue. Additionally, in analyzed samples, no histological signs of inflammation were detected. Cytokine profile expression did not differ between preclinical and clinical stages of the disease, as well as in the serum of wild-type mice.
Conclusions: Morphofunctional disturbances in the peripheral sciatic nerve do not correlate with changes in circulating blood cytokines in ALS mice. Evaluation of changes in the peripheral nerve may be a diagnostic hallmark for the development, stage of the disease and the effectiveness of the proposed therapy. This work was supported by the RFBR grant № 18-44-160029.

54ASM-0308 | Silymarin, boswellic acid and curcumin enriched dietetic formulation reduces the growth of inherited intestinal polyps in an animal model

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Aim. To assess whether an enriched nutritional formulation (silymarin, boswellic acid and curcumin) with proven anti-carcinogenetic properties may prevent inherited intestinal cancer (IC) in animal model.
Methods. Forty adenomatous polyposis coli multiple intestinal neoplasia - ApcMin/+ mice were used for the study of cancer prevention. They were divided into two groups: 20 assumed standard and 20 enriched diet. At the 110th day animals were sacrificed. In each group, four subgroups received intraperitoneal bromodeoxyuridine (BrdU) injection at different times (24, 48, 72 and 96 hours before the sacrifice) in order to assess epithelial turnover. Moreover, we evaluated the following parameters: intestinal polypoid lesion number and size on autoptic tissue, dysplasia and neoplasia areas by histological examination, inflammation by histology and cytokine mRNA expression by real-time polymerase chain reaction,, BrdU and TUNEL immuno-fluorescence for epithelial turnover and apoptosis, respectively. Additionally we performed western blotting analysis for the expression of estrogen alpha and beta receptors, cyclin D1 and cleaved caspase 3 in normal and polypoid tissues.
Results. Compared to standard, enriched diet reduced the total number (203 versus 416) and the mean number $\pm$ SD/animal (12.6 $\pm$ 5.0 versus 26.0 $\pm$ 8.8; $P < 0.001$) of polypoid lesions. In enriched diet group a reduction in polyp size was observed ($P < 0.001$). Histological inflammation and pro-inflammatory cytokine expression were similar in both groups. Areas of low grade dysplasia (LGD; $P < 0.001$) and intestinal carcinoma (IC; $P < 0.001$) were significantly decreased in enriched diet group. IC was observed in 100% in standard and 85% in enriched formulation assuming animals. Enriched diet showed a faster epithelial migration and an increased apoptosis in normal mucosa and LGD areas ($P < 0.001$). At western blotting, ER beta protein was well expressed in normal mucosa of enriched and standard groups, with a more marked trend associated to the first one. ER alpha was similarly expressed in normal and polypoid mucosa of standard and enriched diet group. Cleaved caspase 3 showed in normal mucosa a more strong signal in enriched than in standard diet. Cyclin D1 was more expressed in standard than enriched diet group of both normal and polypoid tissue.
Conclusions. Our results are suggestive of a chemo-preventive synergic effect of the components (silymarin, boswellic acid and curcumin) of an enriched formulation in inherited IC. This effect may be mediated by the reduction of epithelial proliferation, the increase of apoptosis and the acceleration of villous cell renewal due to dietary formulation intake.

54ASM-0333 | The role of autophagy in tyrosine kinase inhibitors treatment of lung cancer depending on the presence of different EGFR gene mutations.

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Background: One of the main causes of tumor formation in lung cancer is the appearance of mutations in the EGFR gene, which lead to its constant activation, independent of the ligand presence. For the treatment of such tumors, drugs that inhibit the kinase activity (TKIs) of EGFR by binding to the active center of the receptor are used. However, after a few months of treatment, additional mutations in the EGFR gene appear, causing resistance to the therapy. One of the mechanisms of this resistance may be activation of autophagy processes in these cells. So the aim of the work was to assess autophagy level and its role in TKIs therapy of the lung adenocarcinoma cells with different EGFR status.

Materials and Methods: In this study we used human non-small cell lung cancer (NSCLC) cell line H1299 (wild-type EGFR). Using the CRISPR/Cas9 gene editing system, we introduced specific point mutations in 20 and 21 exons of the EGFR gene both as single mutations (H1299 T790M and H1299 L858R) and as double mutations (H1299 L858R/T790M). H1975 (L858R/T790M) and H1650 (19 exon deletion (E746-A750)) were also used as NSCLC lines with EGFR mutations that were not genomically edited. As TKI the Gefitinib was used. Cell survival during treatment was evaluated using an MTT test. The level of autophagy was determined using Western blot analysis, immunocytochemistry and electron microscopy. Cell images were obtained using a confocal microscope Olympus FV3000 (Olympus Corporation, Germany).

Results: Autophagy processes were found to be actively involved in the catabolism of lung cancer cells in all H1299 cell types. However, the highest level of autophagy was observed in cells with an activated form of EGFR (H1299/L858R). In H1975 and H1650 cells, the baseline level of autophagy was lower than in H1299. However, after stopping the terminal stages of autophagy using the drug Chloroquine, the amount of LC3-II (autophagy marker) was significantly increased in H1650 cells, which may indicate faster processes of autophagosome formation and degradation in H1650. Exposure to the drug Gefitinib led to an increase in autophagy in all the studied lines, except for H1975.

Conclusions: NSCLC cells that are sensitive to TKIs therapy (H1299/L858R, H1650) have a higher level of autophagy, than resistant cells. Autophagy is not an adaptive mechanism for H1975 cells in antitumor therapy with the drug Gefitinib.

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54ASM-0344 | The effect of bovgialuronidase azoximer (Longidaza®) on antibiotics susceptibility of biofilm-embedded bacteria in vitro

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Background: Since ancient times increasing the efficacy and speeding up both chronic and acute wounds healing and burns are important challenges in clinical medicine. Proteolytic enzymes are widely known as efficient tools for tissue recovery as they degrade damaged cells, necrotic material, mediators and toxic products thereby decreasing the wounds healing time, edema and pain.

Materials and Methods: The ability of the bovgialuronidase azoximer (Longidaza®) to destroy bacterial biofilms of S. aureus, E. faecalis and E. coli, as well as the effect of the combined application of Longidaze with antibiotics using the drop plate analysis bacterial viability in biofilms are studied

Results: This is shown that two hours treatment with 750-1500 international units of Longidaze leads to a twofold decrease of the biomass of mature biofilms formed by *E. faecalis*, *E. coli* and to a lesser extent *S. aureus*. Along with that bovgialuronidase azoximer does not inhibit the formation of bacterial biofilms. When applied in combination, bovgialuronidase azoximer significantly increases the efficacy of following antibiotics against biofilm-embedded bacteria: ciprofloxacin and amoxicillin against *E. faecalis*; cefuroxime, phosphomycin, ciprofloxacin and amikacin against *E. coli* and cefuroxime against *S. aureus*.

Conclusions: Thus, bovgialuronidase azoximer increases infiltration of antibiotics to bacterial cells in biofilm thereby allowing enhancing their efficiency, reducing the dose and side effects of antimicrobials.

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54ASM-0345 | Competition between the N-and C-terminal regions of the small heat shock IbpA protein from Acholeplasma laidlawii controls its oligomerization pattern and chaperone-like activity

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Background: Small heat shock proteins (sHSPs) are ubiquitous molecular chaperones that prevent irreversible denaturation of proteins. While Escherichia coli has two sHSPs IbpA and IbpB which work closely together, a free-living Mollicute Acholeplasma laidlawii carries only one gene encoding for the sHSP AlIbpA

Materials and Methods: analytical size-exclusion chromatography; chemical cross-linking by glutaraldehyde; pull down assay; surface plasmon resonance assay; chaperone-like activity assay by SYPRO Orange; transmission electron microscopy.

Results: In vitro, regardless of temperature, AlIbpA forms a heterogeneous mixture of approximately 24-dimensional globules, fibrils, and huge protein aggregates. Removal of 12 or 25 N-terminal amino acids resulted on fibrils formation and enhanced the protein's ability to prevent temperature-induced insulin aggregation by taking the fibrillar form as the active protein. The deletion of the C-terminal motif or replacement of the C-terminal LEL motif with SEP reduced the temperature stability of AlIbpA and completely eliminated its chaperone function, although the protein remained predominantly in the globular state. This suggests that the C-terminal LEL motif is necessary for the chaperone-like activity of AlIbpA and the formation of fibrils. Double N-and C-terminal truncations destroyed both chaperone-like activity and the formation of huge oligomers. Since the globular form of sHSPs is considered as their inactive form, our data suggest that the N-terminal part of AlIbpA is responsible for the formation of huge globules (the low-active form) and behaves as an intramolecular inhibitor of fibrillation (the active form) and substrate binding.

Conclusions: Taken together, these data demonstrate non-trivial properties of AlIbpA, in which the competitive action of N - and C-terminal molecules regulates the balance between fibrillar or globular structures that represent a possible molecular mechanism for regulating AlIbpA activity.

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54ASM-0346 | Development of nanocomplexes for enzyme controlled drug delivery

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Background: Targeting of the precise molecular mechanisms underlying tumour growth and development has become of the key strategies for drug development. However, most of these drugs demonstrate serious side effects due to the off-target activity. The next step in this paradigm would be research of the novel multifunctional agents, capable of specific release of the therapeutics only in the precise tumour microenvironment. Nanoparticles are one of the most promising platforms for development of such agents due to their physical, chemical and biological properties. Their surface could be easily modified with various types of biomolecules, that can bind specific targets on the cell surface or degrade under certain conditions. In this connections one of the most appealing markers for recognition of tumour microenvironment are MMP7 and MMP9 proteases.

Materials and Methods: We assembled a multilayer nanostructure that utilized 1 um magnetic microspheres as the core (Merck Millipore, France) with 100 nm polymer nanoparticles immobilized on its surface. The model antitumour drug was also immobilized on the surface of the core. As the interface for enzyme recognition and a link between core and surface particles we target peptides for thrombin and MMP7, that were specifically broken down by model peptidases. Upon addition of the enzyme, the system changed its configuration, which was supported by scanning electron microscopy (SEM), and the surface of the core became exposed to the environment.

Results: Here we show the new smart enzyme-responsive nanoparticle-based system as a potential tool for biomedical applications. The system specifically disassembled in the presence of the thrombin or MMP7 protease, thus making the model drug molecule on the core surface available for its target. This result was supported by SEM analysis and peroxidase target-binding assay. Its satisfactory performance was observed both in the buffer system and in 10% FBS, which served as the model for physiological liquids.

Conclusions: The proposed system is an attractive platform for development of enzyme based targeted drug delivery and biosensing. Different parts of the reported study were supported by Russian Fund for Basic Research, project number 18-03-01252.

54ASM-0359 | Cytotoxicity of potassium salts of terpenic acids conifer oleoresins

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Background: Terpenes are the largest and the most diverse class of natural plant products with a wide range of biological activity. A number of studies provide data on the antitumor activity of terpenes, their effectiveness in chemoprophylaxis and cancer chemotherapy. Given the high potential of terpenes as drug components, a comprehensive study of the toxicity of different terpenes and their derivatives in respect to normal and tumor cell lines is needed.
Materials and Methods: Norway spruce (*Picea abies L.*), Siberian stone pine (*Pinus sibírica Du Tour*), Siberian fir (*Abies sibirica Ledeb.*) and Scots pine (*Pinus sylvestris L.*) were used as starting materials. Samples of oleoresin were taken using the sedimentation method in the Zmeinogorsky District of Altai Krai (Russia). Depending on the oleoresin used, odourless crystalline products ranging from light yellow to light brown were obtained. The salts obtained were hydrolytically stable.
Results: According to GC-MS analysis, the main component of oleoresin in the studied plants was abiotic acid: its content was 50% or more of the total number of identified compounds.
Toxicity of potassium salts of terpenic acids was investigated using the Cell Viability BioApp protocol of the Cytell cell imaging system in relation to conditionally normal cell line Wi38 VA 13 subline 2RA (cells of a human lung embryo) and tumor lines - MCF-7 (human breast adenocarcinoma cell line), M-Hela - (human cervical carcinoma cell line). In a wide range of studied concentrations of 0.000225-0.125% potassium salts of terpenic acids of oleoresin showed high cytotoxicity against conditionally normal cell line and relatively low cytotoxicity against tumor lines. Thus, the viability of cells of the lung embryo, after treatment with a solution of Siberian fir potassium salt at a concentration of 0.0019% was more than 4 times lower than the studied tumor cells.
Conclusions
Potassium salts of terpenic acids of oleoresin of a number of coniferous plants did not show specific cytotoxicity in relation to the investigated tumor cell lines. It is advisable to continue research on the biological activity of drugs and the prospects for their use as disinfectants for external use.

S8 – CLINICAL ULTRASONOGRAPHY: TIPS & TRICKS

54ASM-0405 | Rat prostate: practical tips for ultrasonographic monitoring

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Background: Prostate is the largest accessory gland of the male reproductive tract. The prostate of men over 40 years-old is frequently affected by several pathologies, like benign prostate hyperplasia and cancer. Rats have been used as model to study prostate cancer. This study intended to address the usefulness of ultrasonography for rat prostate monitoring.
Materials and Methods: Eight male Wistar Unilever rats were acquired from Charles River Laboratories and maintained under controlled conditions of temperature, humidity, air system filtration and light/dark cycle. The prostate was evaluated by ultrasonography in awake animals. The animals were restrained by a researcher and placed in supine position. The skin of the inguinal region was shaved using a machine clipper (AESCULAP® GT420 Isis, USA). A real-time scanner (Logic P6®, GE, USA) and a 12 MHz linear transducer were used. Acoustic gel (Parker Laboratories Inc., USA) was applied. A complete transverse scan using B mode was performed from the cranial to the caudal region of the prostate, and a sagittal scan was performed moving the probe from the right to the left side. Procedures were approved by the Portuguese Ethics Committee (no.021326).
Results: Prostate was easily evaluated by ultrasonography in all animals. In the transverse scan, the urinary bladder presents as a round to oval shape filled with urine (anechoic structure) and the prostate lobes were visible around it. The ventral prostate lobes appear as hypoechoic elongated structures (one right and one left) with a hyperechoic capsule, placed ventrally to the urinary bladder. In this scan, the dorsal prostate was observed close to the urinary bladder neck, as a round hypoechoic structure with a hyperechoic capsule, dorsally to the urinary bladder. In the sagittal scan, the urinary bladder was observed as an elongated structure filled with urine (anechoic content). The ventral prostate lobes were occasionally observed ventrally to the neck of the urinary bladder, as previously described. The dorsal prostate was observed dorsally to the neck of the urinary bladder, presenting as a round to elongated shape, with a hypoechoic appearance and a hyperechoic capsule.

Conclusions: The ultrasonography is a non-invasive and accessible tool for prostate monitoring in the rat model.

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S9 – BIOINFORMATICS & COMPUTATIONAL BIOLOGY FOR BIOMEDICINE

54ASM-0249 | Rotation and propulsion in 3d active chiral droplets

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Background: Bio-inspired materials play a fundamental role in the development of novel devices for drug-delivery. Such systems are usually composed by a large number of constituents capable of converting energy coming from ATP hydrolysis into motion. The mutual interaction between the collective behavior of active agents (such as microtubule bundles activated by means of kinesin motors) and the hydrodynamic feedback of the underlying fluid gives rise to a plethora of collective behaviors, not observable in absence of activity. In the last decade a number of experiments have shown that both dense bacterial suspensions and cytoskeletal suspensions can develop a dynamical ordered state commonly addressed as 'spontaneous flow' where the constituents are able to autonomously generate self-sustained flows.

Materials and Methods: In this seminar I will focus on the Physics of active fluids -for instance bacterial or cytoskeletal suspensions- making use of the theory of active liquid crystal. This is a well validated approach to capture the dynamics of active suspensions and consists of describing the hydrodynamic state of the system with continuous fields. By means of numerical simulation, we investigated the behavior of a droplet of intrinsically chiral active Liquid Crystals suspended in a passive background matrix varying the intensity of active forcing -a quantitiy experimentally related to the concentration of ATP.

Results: First, we found that active forcing is able to sustain a stable rotational motion of droplets with low or vanishing cholesteric strength. As the chirality is increased, the feedback effect between elasticity and activity push the droplet into an asymmetric configuration, known as Frank-Pryce structure, enabling a new motility mode, where the rotational motion of surface defects is converted into propulsion. The velocity of the migration can be triggered by varying the intensity of the active forcing. Second, by considering an active chiral stress, we found the mirror rotation of two pairs of disclination lines exhibiting a *coiling and relaxing* dynamics leading to periodical behavior. Furthermore we explore the effects on the flow and the chiral pattern as the ratio between the radius of the droplet and the cholesteric pitch increases.

Conclusions: Here we present a study the effect of activity on a droplet of chiral matter, finding a surprisingly rich range of dynamical behaviours, ranging from spontaneous rotations to screw-like motion. The phenomena we uncover require both chirality and activity that may be exploited for the development of new smart materials and devices for drug-delivery.

54ASM-0280 | Non-supervised analysis of neurotoxicity of mitochondrial poisons 6-OHDA and rotenone

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Background: Neuronal cells are highly dependent on mitochondria-generated ATP for cell growth, survival and function, and small disturbances on mitochondrial activity and morphology could lead to a phenotype of neurodegeneration and cell death. To understand the causes and consequences of neurodegeneration, and since to assess the crosstalk between different measures, conventional statistical analysis is often insufficient, our aim was to use unsupervised machine learning tools. Principal Component Analysis (PCA) and clustering methods are nowadays pivotal to unveil hidden patterns and to find groups with similar properties.

Materials and Methods: To induce different levels of neurodegeneration, differentiated human SH-SY5Y neuroblastoma cells were treated with increasing, non-lethal concentrations of either rotenone or 6-hydroxydopamine. Mitochondrial superoxide anion was evaluated by following Mitosox Red oxidation, while Mitotracker Red fluorescence area and intensity were used to assess mitochondrial morphology and membrane potential. Cell death was followed with Caspase-Glo 3/7 Assay. Exploratory data mining was carried out using

Orange biolab. Unsupervised machine learning approaches like PCA, K-means and hierarchical clustering were used. Correlation measures and feature selection techniques were also applied, establishing a subset of attributes that can be used in the future development of a predictive model.

Results: The first two PCA components cover around 70% of sample variance. A visual inspection of the scatter plot reveals a clear separation between the treatments. Moreover, PC1 alone achieves a reasonable global separation between rotenone and dopamine treatments, failing just in the differentiation of smaller concentrations and control samples. K-means produces cohese and homogeneous subsets. In detail, we observed that it is possible to cluster different rotenone concentrations and to distinguish the higher dopamine concentrations from controls. Conversely, smaller dopamine and rotenone concentrations, as well as control samples, tend to be clustered together. Hierarchical clustering displays similar outcomes, clearly differentiating the three conditions under study.

Finally, the application of Pearson correlation, feature selection and information gain techniques reveal that Mitotracker Red area and intensity are the most promising features to be used in the development of a predictive model.

Conclusions: Unsupervised machine learning methods are able to distinguish between different cell treatments and control cells. Also, they identify promising features to be used in the development of predictive models to understand the causes and consequences of neurodegeneration.

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54ASM-0339 | Exploratory data analysis applied to metabolic profiling of urine-derived stem cells from young and elderly women

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Background: There is increasing evidence supporting that aging-related systemic bioenergetic decompensation in the human body is a primary mediator of chronic metabolic diseases. The principal causes of bioenergetic decompensation are linked with impaired substrate oxidation and oxidative phosphorylation related to mitochondrial dysfunction. Given that urine-derived stem cells (UDSC) carry the donor´s genetic background and are non-invasively collected, UDSC from young and elderly female donors were characterized in term of their mitochondrial oxygen consumption and indirect glycolytic activity, to evaluate whether aging affects cell and mitochondrial energy metabolism in this particular type of cell.

Materials and Methods: UDSC were isolated and divided in two groups (young adults: 22-35 years old, n = 13; elder adults: 70-94 years old, n = 10). Mesenchymal and hematopoietic stem cell markers were studied by flow cytometry. Oxygen consumption (OCR) and extracellular acidification (ECAR) rates were measured using Seahorse XF Cell Mito and Glycolysis Stress assays, respectively, using a Seahorse XFe96 Extracellular Flux Analyzer. Data were statistically analyzed using unpaired *t*-test or Mann-Whitney test. Exploratory data analysis computational tools were applied to determine a minimal subset of features that allow for a separation between the two groups.

Results: Both UDSC from young and elder female donors were positive for CD44, CD73, CD24, CD90 and CD105, proving their mesenchymal stem cell nature. In terms of bioenergetic capacity, USC from elder donors were found to have higher mitochondrial maximal respiration levels and bioenergetic health index (BHI), when compared to UDSC derived from young adults. Exploratory data analysis revealed that OCR-related features tend to have increased ability to differentiate the groups. Just by considering the basal and maximal respiration levels, together with the BHI index, K-means was able to find clusters with a purity of 70% to 75%. To further improve the results, we engineered two new features: RGlyco (glycolytic reserve/glycolytic capacity), and RPotential (% Baseline ECAR/% Baseline OCR). Repeating the application of K-means with these two new features and BHI lead to an enhanced purity of 80%. A hierarchical clustering method with complete linkage was applied to the same data and achieved similar results.

Conclusions: The application of bioinformatics tools to these data revealed promising results, based on bioenergetic capacity, to differentiate USDC from young and older female donors. USDC are promising cell platforms for drug development and to investigate metabolic remodelling during aging.

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54ASM-0352 | A Data Science Approach to Sub-chronic Doxorubicin Cardiotoxicity Reveals Hidden Patterns of Metabolic Remodeling

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Background: Doxorubicin (DOX) is one of the most potent antineoplastic drugs. Despite possessing a superior anti-cancer activity, a broader clinical use of DOX is limited by a dose-dependent and cumulative cardiomyopathy involving deterioration of mitochondrial function, leading to the development of cardiomyopathy and ultimately congestive heart failure. Nowadays, data driven approaches aimed for a deeper analysis of biological datasets are very effective to uncover additional information that may have been missed by the use of traditional statistical methods. Still, these approaches are not very common.
Materials and Methods: We used a sub-chronic animal of DOX cardiotoxicity (7 weekly injection) in which hearts from saline and DOX-treated rates were perfused ex vivo with two different substrates (glucose and galactose plus glutamine-GG). Hemodynamic parameters were titrated with three different metabolic inhibitors (iodoacetate, cyanide and rotenone), in order to identify metabolic alterations. These data were then processed through different techniques and computational tools, including Machine Learning (ML) Algorithms in order to expose possible hidden patterns, focusing exploratory methods, such as PCA, correlation analysis and decision trees.
Results: Using glucose as substrate, the DOX-treated group showed a better tolerability to inhibitors than SAL. The presence of inhibitors in the perfusion also generally decreased the total amount of proteins detected by Western Blotting, although glycolytic proteins were increased when hearts were perfused with glucose, contrarily to GG perfusion. The results suggest that DOX-treated rats suffered a metabolic remodeling resulting on stronger glycolytic fluxes to maintain contractility, although no obvious mitochondrial defect was uncovered. Thus, we confirmed that DOX treatment remodels cardiac metabolism. Our computational analysis figured out the relationships between each feature, showing that the glucose-perfused hearts inhibited with iodoacetate had a different behavior than the remaining combinations, with most differences found between treatments, suggesting that this experimental condition is the most promising to better understand sub-chronic DOX effects.
Conclusions: Accordingly, this work also proves that a detailed data analysis driven by ML allows a better exploration of this biological dataset enabling new discoveries and breakthroughs in this field.
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54ASM-0362 | Detecting treatment-response heterogeneity in cancer cell populations

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Ex vivo drug-sensitivity assays are a basic component of biomedical research. Typically, cells are treated with varying concentrations of drug and the number of viable cells is measured at one or more fixed time points. The resulting data are normalized and fitted to produce viability curves, whose characteristics (e.g. IC50) are evaluated to compare drug sensitivity across multiple drugs or cell populations. Increasingly, such drug screens are used as a tool in personalized medicine to evaluate and rank the potential efficacy of therapeutic agents on a patent's disease cell population. However, the interpretation of cell viability curves and associated metrics are confounded by the presence of cellular heterogeneity within the population. For example, the presence of subclones of drug-resistant cells within an otherwise drug-sensitive population may shift the overall drug response viability curve, resulting in an intermediate drug sensitivity profile that does not accurately represent any single individual in the population. Under treatment, the initially overlooked drug-resistant subpopulation may expand while drug-sensitive cells are depleted, thus driving population rebound dynamics that cannot be predicted by this viability curve.
In this work, we develop mathematical, statistical and computational tools for detecting the presence of cellular subpopulations with differential drug responses, using standard cell viability assessment data at the total population level. Our method estimates the number and mixture frequencies of distinct phenotypic components in the composite population as well as the drug-response profile of each subpopulation. It uses an underlying population dynamic model to describe the evolution of individual subclones over time. We show that this model accurately recapitulates experimental raw viability data using non-small cell lung cancer and lymphocytic leukemia cell lines with differential responses to the specific drugs. Then, we validate the methodology on viability data from admixtures of these cell populations at various known frequencies. Finally, we explore the usefulness of the method

to estimate drug response heterogeneity in different leukemia patient samples.

54ASM-0376 | In silico analysis of SARS-CoV-2 spike glycoprotein and insights into antibody binding

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Background: The purpose of the study was to find antibodies against SARS-CoV-2 infection.
Materials and Methods: In silico analysis, structural bioinformatics and computational biology were used to modify SARS-CoV antibodies to bind against SARS-CoV-2. Chimera software, Swiss-Model, Rosetta Dock and Bridges, Stampede2 and Frontera supercomputers were used to achieve this goal.
Results: Three antibodies that bind to the receptor binding domain (RBD) of ACE2 (Angiotensin Converting Enzyme 2) in SARS-CoV were modified (mutated) to achieve binding to SARS-CoV-2 receptor binding domain. Structural modeling of SARS-CoV-2 RBD provided a structure for antibodies to bind according to pdb structures for 80R, m396 and S230 antibodies. The structures were analyzed at atomic detail, mutations were introduced to maximize binding and docking experiments were run with Rosetta Dock and Rosetta SnugDock to find the best mutations that achieve the goals of binding. In addition, a model of SARS-CoV-2 infection was built in Bridges and Frontera Supercomputers which is the BioArt winner of this year 2020 to provide insights on how to fight the COVID-19 disease.
Conclusions: The three antibodies were mutated and found that 7 mutations for 80R, 5 mutations for m396 and 3 mutations for S230 were enough to achieve binding similar to SARS-CoV. The mutations for 80R focused on improving aromatic-aromatic interactions, for m396 focused on electrostatic interactions and for S230 on a combination of both interactions. The mutations were introduced after careful analysis at atomic detail that pointed to those kind of residues. These in silico made antibodies can be very useful to fight COVID-19 as passive immunity provided to patients. These antibodies have to be tested in the bench and then in clinical settings therefore this is an initial step on finding therapeutics against SARS-CoV-2. Finally, this approach can be used to find more antibodies that can fight the disease.

54ASM-0406 | Data Extraction from Mitochondrial Motility Tracking using Computational Algorithms

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Background: Study of mitochondrial motility has gained increasing interest in recent years and live cell imaging is crucial to better understand the underlying physiological factors. Different strategies have been employed to track mitochondrial movement, particularly to investigate mitochondrial transport in neuronal axons.
Materials and Methods: This work used an open source automated MATLAB model, developed by Judith Kandel, Philip Chou, and David M. Eckmann, describing mitochondrial motility as a lognormal distribution which provides a quantitative paradigm to assess mitochondrial movement. Differentiated SH-SY5Y cells were treated with 31.25 nM rotenone and 6.25 μM 6-hydroxydopamine for 24 h. Mitochondria were then labelled with 25 nM Mitotracker Red and ten-minute movies of one frame per second were obtained under a Nikon Ti-E H-TIRF microscope. Then, the videos were preprocessed in ImageJ as time-lapse images. First, they were convolved, then converted to the frequency domain using a Fast Fourier Transform (FFT) and subjected to a bandpass filter ranging from 2 to 100 pixels. Finally, the resulting images were manually thresholded to best eliminate the noise. The resulting stacks of images were analysed by the selected model focusing cell projections. After processing and comparing each frame, the model output two histograms of log values of net, the total distances traveled by all mitochondria objects found and the respectively, trajectories. In addition, the TrajPy python package was used to further quantify the trajectories by estimating a set of features: the mean velocity, anisotropy, straightness, efficiency, among others. These features helped to highlight relevant differences between the control and the treated samples.
Results: We found that mitochondria in treated cells appear to travel less in average compared to controls which indicates mitochondrial dynamics is affected by the treatments. This decrease in mobility is more accentuated in rotenone-treated cells and came along with an increase in trajectories anisotropy and straightness. Strikingly, the treated mitochondria

presented more regular movement when compared to the control, which contrasted with their smaller range of velocities.
Conclusions: Moreover, we confirmed that the combination of the selected open source models provides an effective method to quantify mitochondrial motility, thus allowing its application in future studies using different cell treatments and conditions.
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54ASM-0407 | Exploratory analysis of mitochondrial metabolism in lymphoblasts from Amyotrophic Lateral Sclerosis patients to reveals discriminant biomarkers

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Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative motor disease with no current treatment, with a life expectancy of 2–5 years after diagnosis. Substantial evidence indicates that mitochondrial dysfunction contributes decisively to neurodegeneration in ALS. New methods are needed for patient stratification to find fingerprints and therapeutic targets for ALS based on metabolic biomarkers.
Materials and Methods: We used a total of 9 lymphoblast cell lines obtained from patients with known mutation SOD1 (mutSOD1) or unknown mutation and age/sex matched controls. Three cohorts were considered, consisting in one cell line of each group for 46y old females (C1), 46y old males (C2) and 26y old males (C3). We obtained experimental data from mitochondrial respirometry, using MitoStress and ATP Rate tests in a Seahorse XFe96 Extracellular Flux Analyser, and metabolic profiling, using Biolog Mitochondrial Phenotypic Microarrays. For the computational data analysis, Python 3 version 3.7.3, Pandas and SciPy packages were used. Correlation matrices and cluster maps were plotted using Matplotlib and Seaborn modules. The information was grouped by a two-way hierarchical clustering method using the squared Euclidean distance metric for both dendrograms. Estimation of the mutual information between each individual feature and the target (information gain) and decision trees were obtained with Orange Biolab.
Results: Exploratory analysis evidenced differences in metabolic phenotype between lymphoblasts from ALS patients with and without known mutSOD1, compared to their healthy age-matched counterparts. Lymphoblasts from ALS patients presented mitochondrial alterations and can be used to discern mutSOD1 effects on mitochondrial function. Clustering analysis using only the Seahorse measurements with highest information gain for the groups with the same age (C1 and C2): XF ATP Rate Index, Stressed ECAR, glycolytic ATP Rate and mitochondrial-ATP Rate provided a perfect separation between target groups, with mutSOD1 segregating into a more distinct cluster, indicating that these were better distinguished from controls than patients with unknown mutation. Applying tree-based models to Biolog Microarrays results allowed distinguishing ALS patients with known and unknown mutSOD1 from controls based only in two features: ketobutyric acid and acetyl-L-carnitine plus malic acid.
Conclusions: Computational data analysis revealed promising biomarkers to discriminate between two types of ALS patients and sex/age matched control, that can be used in the future as therapeutic targets for ALS drug development.
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S10 – KIDNEY BETWEEN HEALTH AND DISEASE

54ASM-0185 | Innovative Functional Food Based on Apulian Lens culinaris for Contrasting Sarcopenia in Dialysis Patients (ALTIS)

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Background: Sarcopenia, the loss of skeletal muscle mass and strength typical of older people, is commonly present in dialysis patients, due to clinical and nutritional conditions. Sarcopenia is characterized by malnutrition, progressive protein/energy depletion, poor quality of life, health complications and increased mortality risk. The purpose of the ALTIS study is to validate a dietary supplement, composed by vegetable proteins and fibres, to reduce sarcopenia in dialysis.
Materials and Methods: Eating habits, protein and fiber intakes of a group of 15 dialysis patients have been evaluated by a food frequency questionnaire. A randomized pilot study was designed, with inclusion/exclusion criteria, study protocol, outcomes and methods of assessment. Under the ALTIS project, a supplement based on Altamura *Lens culinaris* IGP flour, vegetable proteins and essential micronutrients is being produced for clinical validation.
Results: Nutrients assumption in dialysis patients resulted significantly below the reference daily intake for proteins

(1.2 g/kg/die) and fibres (35 g/die). Medium intake of proteins for males and females was 74% and 56% than recommended, respectively. Average fibre intake was 32% than recommended. A two-arms prospective, randomized, open-label, controlled pilot study was designed and inclusion/exclusion criteria were defined. Twenty dialysis patients will be randomized for dietary supplementation or no intervention for three months. Primary outcomes: change in muscle mass evaluated by bioimpedance analysis. Secondary outcomes: change in nutritional status, anthropometry, gastrointestinal symptoms, metabolic parameters, serum levels of microbiota-derived uremic toxins, intestinal permeability and inflammation. The protocol was approved by the Ethics Committee (Policlinico, Bari, Italy) and registered on ClinicalTrials.gov with ID NCT04223206.

Conclusions: Protein and fibres intake in dialysis patients are below the recommendations. The approved pilot trial will validate the efficacy of the ALTIS supplement in reducing sarcopenia and improving clinical, nutritional and experimental parameters. Funding: project code ALTIS - K2DTD75, Regione Puglia 'INNONETWORK 2017'.

S11 – STEM CELLS & CELL THERAPIES

54ASM-0084 | The effect of mesenchymal stem cells with overexpression of cytokines on the viability of human melanoma

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Background: Currently, immunotherapy (in particular, cytokine-based therapy) of cancer is considered as a promising method to treat various types of tumors. Cytokines and chemokines play an important role in the processes of selective destruction of tumor cells, while not affecting healthy cells of the body. Mesenchymal stem cells (MSCs) can become an ideal vector for delivering cytokines to the tumor microenvironment since they exhibit a homing behavior toward tumor sites.

Materials and Methods: Human MSCs were isolated from adipose tissue. The cells were largely positive for MSC surface markers including CD29, CD44, CD73, CD90 and CD105 and negative for haematopoietic stem cell surface markers. MSCs were transduced with recombinant lentiviral vectors encoding tumor necrosis factor ligand superfamily member 10 (TRAIL), interferon alpha-17 (IFNA17), interleukin-2 (IL2), granulocyte macrophage colony-stimulating factor (GM-CSF) or reporter red fluorescent protein (RFP). The gene and protein expression was confirmed by qPCR and Western blot analysis. To evaluate the influence of MSCs with overexpression of various cytokines on the melanoma M-14 cell viability M-14 cells were co-cultured with native and genetically modified MSCs in 1:1 ratio (5×10^4 cells of each type) for 72 h. Viability of M-14 cells were determined using Annexin V assay.

Results: Co-culture of M-14 cells with MSCs-TRAIL (79.1 ± 0.42%), MSCs-IFNA17 (78.0 ± 5.3%) and MSCs-IL2 (84.45 ± 7.5%) resulted in significant decrease in cell viability as compared to that when tumor cells were co-cultured with native MSCs (93.3 ± 0.42%) or MSCs-RFP (91.75 ± 0.49%). At the same time co-culture of M-14 cells with MSCs-GM-CSF increased the cell viability compared to control co-cultures.

Conclusions: The use of MSCs-TRAIL, MSCs-IFNA17 or MSCs-IL2 can be effective in the treatment of melanoma. However, further studies of MSC efficiency in animal tumor models are required. This study was supported by grant from the Russian Foundation for Basic Research grant 18-04-01133 and the Russian Government Program of Competitive Growth of KFU.

54ASM-0086 | Analysis of the antitumor activity of extracellular vesicles isolated from mesenchymal stem cells with overexpression of cytokines and tumor suppressors

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Background: Almost all human cells release extracellular vesicles (EVs) participating in intercellular communication. EVs are rounded structures surrounded by the cytoplasmic membrane, which embody cytoplasmic contents of the parent cells. Since the orientation of surface receptors persists during EV formation, it is EVs that are of interest as a therapeutic tool for cell-free therapy that has targeted delivery. The ideal cell type for the EV production is mesenchymal stem cells (MSCs) as they exhibit a homing behaviour to tumor niches.

Materials and Methods: In this study, human MSCs were isolated from adipose tissue and genetically modified to overexpress tumor necrosis factor ligand superfamily member 10 (TRAIL), interferon alpha-17 (IFNA17), interleukin-2 (IL2), granulocyte macrophage colony-stimulating factor (GM-CSF), phosphatase and tensin homolog deleted on chromosome 10 (PTEN) or red fluorescent protein (RFP). Extracellular vesicle release from native and genetically modified MSCs was induced by cytochalasin B treatment. Cytochalasin B is a substance that interferes with the

polymerization of actin filaments, causing disruption of the cytoskeleton. To evaluate antitumor properties 5×10^4 HCT-116 human colon carcinoma cells were cultured with 20 μg/mL EVs isolated from native and genetically modified MSCs. HCT-116 cell viability were determined using Annexin V assay.

Results: Cultivation of HCT-116 cells with EVs-TRAIL (78.85 ± 0.77%), EVs-IFNA17 (77.5 ± 2.26%) resulted in significant decrease in cell viability as compared to that when tumor cells were cultured with EVs from native MSCs (82.1 ± 1.41%) or EVs-RFP (84.9 ± 0.42%). At the same time cultivation of HCT-116 cells with EVs-GM-CSF (83.95 ± 0.21%), EVs-IL2 (84.45 ± 7.5%) and EVs-PTEN (86.65 ± 0.63%) increased the cell viability compared to control cultures.

Conclusions: The use of EVs-TRAIL and EVs-IFNA17 can be effective in the treatment of colon carcinoma. However, further studies of EV efficiency in animal tumor models are required. This study was supported by the Russian Science Foundation grant 18-74-10044 and the Russian Government Program of Competitive Growth of KFU.

54ASM-0095 | The effect of mesenchymal stem cell derived extracellular vesicles on tumor spheroids in vitro

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Background: Mesenchymal stem cells (MSCs) have a tropism for tumor microenvironment (TME), and therefore, they can be a promising vector for antitumor drug delivery. Nevertheless, MSCs can have undesirable effects, for example oncotransformation, compared to extracellular vesicles (EVs). EVs are membrane structures that play the role of intermediaries between tumor cells and TME because they have the ability to transport lipids, transcription factors, mRNA, and proteins. However, understanding the effect of mesenchymal stem cell derived extracellular vesicles (MSC EVs) on tumor cells in vitro is limited.

Materials and Methods: In this study, colorectal carcinoma cells (HCT-15) were used to create tumor spheroids by hanging drop method. Second passage of adipose derived MSCs were used to isolate EVs. MSC EVs were obtained using 10 μg/mL of cytochalasin B. The addition of MSC EVs to spheroids was carried out at concentrations of 2 μg. The effect of MSC EVs was analyzed using confocal microscopy, flow cytometry, and real-time PCR. The effect of MSC EVs on cell viability was analyzed using Annexin V assay.

Results: After 24 hours of cultivation, using confocal microscopy and flow cytometry the MSC EVs was found to be fused with spheroid cells. The addition of MSC EVs decreased cell viability on the third day of spheroid culture. BCL2 and MMP2 mRNA levels were increased after adding MSC EVs. BCL2 expression was 16.4 fold higher and MMP2 expression was 124.8 fold higher when compared to untreated spheroids.

Conclusions: Thus, it was shown that MSC EVs fused with tumor spheroid cells and reduced their viability, possibly through differentiation-induced apoptosis mechanism. Further research of this mechanism effects is necessary. This study was supported by the Russian Government Program of Competitive Growth of Kazan Federal University and the Russian Science Foundation grant 18-74-10044.

54ASM-0096 | Use of adipose derived stem cells for orthopaedic tissue engineering

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Background: Cell-based therapies and tissue engineering represent potential therapeutic approaches for fractures and bone defects allowing for proper bone repair and regeneration. Adipose derived stem cells (ASCs) are multipotent stem cells that can be easily isolated and induced to differentiate into different mesenchymal lineages; therefore, they are a hopeful cell source for regenerative medicine. The purpose of this investigation was to evaluate the behavior of ASCs cultured on titanium scaffolds (TS) in term of adhesion, proliferation and osteogenic differentiation to demonstrate their successful use for hard tissue engineering.

Materials and Methods: ASCs were seeded on TS and feeded with osteogenic (OM) and growth (GM) media in static conditions. Cell adhesion and proliferation were assessed by MTT test. The osteogenic differentiation was detected with RT-PCR by analyzing the gene expression of some bone markers at different time points from differentiation. Further, the enzymatic activity of alkaline phosphatase (ALP) was biochemically evaluated, the extracellular matrix secretion analyzed with Scanning Electron Microscopy (SEM) and ASCs paracrine effects were investigated.

Results: The MTT assay revealed a good adhesion and proliferation rate of ASCs on TS; SEM analysis showed that cells migrated inside the scaffold and produced an abundant extracellular matrix. After 14 days of culture in OM both the gene expression and the enzymatic activity of ALP were statistically increased. Moreover, the cells grown in conditioned media (CM), showed a different gene expression level of

bone markers respect to ASCs grown in GM, probably due to the ASCs secretome and its paracrine signaling.

Conclusions: Since ASCs were able to adhere to TS and acquire an osteoblastic phenotype, we can conclude that this scaffold/cells construct is effective to regenerate damaged tissue and to restore the function of bone tissue.

54ASM-0098 | Anti-CD19 CAR-T cells show pronounced effect on PC3M-CD19 tumor spheres

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Background: Chimeric antigen receptor T(CAR-T)-cell therapy is a novel type of therapy demonstrating extremely high efficacy in a treatment of hematologic malignancies [Titov *et al.,* 2020]. Recent trials on CAR-T cell therapy of solid tumors haven't been successful due to lack of understanding of how CAR-T cells will act in a heterogenic multicellular environment of solid tumors [Ando *et al.,* 2019]. Tumor spheroids are self-organized stem cell-derived structures, demonstrating histologic similarity with primary tumors. The aim of the study was to evaluate the antitumor efficiency of CAR-T cells on a prostate cancer PC3M spheres.

Materials and Methods: Mononuclears was isolated from blood sample of healthy donor. Selection and activation of T-lymphocytes from a mononuclear fraction were carried out by Dynabeads Human T-activator CD3/CD28. CAR-T cells were produced by lentivirus transduction of T-lymphocytes with CD19-CAR-T-RIAD. PC3M and PC3M-CD19 cells were cultured in spheres media for 5 days. Afterwards, $1x10^6$ of anti-CD19 CAR-T cells were added to test wells. Sphere count and the ratio between tumor and CAR-T cells were accessed after 3 and 24 hours of cell co-culture. Cell viability was measured by 7AAD/Annexin V staining.

Results: Tumor sphere degradation was not observed after 3 hours of CAR-T treatment but pronounced after 24 hours of cell co-culture in PC3M-CD19 model. No differences were observed in plates with PC3M. Intraspheral tumor cell viability was 17.65 ± 0.85% lower in PC3M-CD19 spheres as compared to PC3M, co-cultured with anti-CD19 CAR-T cells for 24 hours. CAR-T cell proliferation was detected in PC3M-CD19 model and accounted for 234.33 ± 12.44%.

Conclusions: CAR-T cells can effectively destroy tumor spheres *in vitro*, further investigation on a mixed cell type 3-dimentional models are required to investigate immunosuppressive features for each cell type, involved in tumor formation *in vivo.*

Work was funded by RSF 19-74-20026 and performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

54ASM-0099 | Apoptotic gene mRNA expression in mesenchymal stromal cells and neuroblastoma cells after co-culturing and cisplatin treatment

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Background: The tumor stroma plays a key role in tumor development, progression, metastasis and the formation of resistance to therapy. Within the tumor microenvironment, stromal cells acquire abnormal phenotypes and functions mediated by complex cell-cell interactions, including direct cell-cell, paracrine factors and other biologically active molecules. We investigated the changes in the mRNA expression level of apoptotic genes BCL2, BAX, BCL2L1, CASP3 after antitumor drug cisplatin (CDDP) treatment of mesenchymal stromal cells isolated from bone marrow (BM-MSCs) and neuroblastoma SH-SY5Y cells in co-culture.

Materials and Methods: To create a co-culture SH-SY5Y (modified by lentivirus encoding green fluorescent protein) and BM-MSCs were mixed in a 1:1 ratio. After 72 hours of co-cultivation CDDP was added (10 μg/mL). After 72 hours of incubation with cisplatin cells were separated by flow cytometry according to the fluorescence spectrum. The expression level of BCL2, BAX, BCL2L1, CASP3 was determined by qPCR, using 18S rRNA as a reference gene.

Results: The mRNA BCL2 expression level in SH-SY5Y after co-cultivation was increased comparing with native cells. The expression level of BAX also increased in whole experimental groups. The BCL2L1 mRNA expression level in SH-SY5Y was increased after co-culturing with CDDP, also the CASP3 mRNA expression level was increased after CDDP treatment. In BM-MSCs the expression level of BCL2 was increased after co-cultivation, but decreased after CDDP treatment of monoculture. The expression level of BAX was increased after co-cultivation with CDDP. The BCL2L1 and CASP3 mRNA expression level in BM-MSCs was increased after CDDP treatment and co-culturing with CDDP.

Conclusions: The data may show the antiapoptotic responses on CDDP treatment and pro-tumor effect of BM-MSCs in co-culture with SH-SY5Y. Study was funded by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities and by the Russian Government Program of Competitive Growth of KFU.

54ASM-0102 | Analysis of antitumor activity of canine mesenchymal stem cells expressing tumor suppressors and immunomodulator genes in vitro

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Background: The use of mesenchymal stem cells (MSCs) modified with tumor suppressor or immunomodulating/antitumor cytokine genes is considered as a new method for the treatment of cancer. Many cytokines and tumor suppressor genes delivered to the tumor site using MSCs have already shown high antitumor activity in cancer animal models. Dogs are an ideal object for studying the behavior and effects of antitumor agent-loaded MSCs in the body. Cancer in dogs demonstrates the same complex interaction of genetics, age and environmental factors as in humans, and these similarities are stronger between humans and dogs than between humans and mice.

Materials and Methods: Canine MSCs were isolated from adipose tissue and genetically modified with lentivirus simultaneously encoding canine tumor necrosis factor ligand (cTRAIL), interferon-β1 (cIFN-β1) and phosphatase and tensin homolog (cPTEN) genes separated by a self-cleaving (Fu)-containing p2A-peptide sequence. The resulting cell line was selected by cultivation with blasticidin S for 10 days. Gene expression was confirmed using qPCR. In order to evaluate the antitumor activity of native and genetically modified (GM) MSCs, 2×10^5 MSCs were cultured for 24 hours, then conditioned medium (CM) was collected. Human colon cancer cells HCT-116 were cultured in fresh RPMI-1640 medium or in CM from native or GM MSCs.

Results: Canine MSCs were largely positive for MSC surface markers including Stro-1, Thy-1, CD10, CD105. MSC multipotency was confirmed by differentiation into chondrocytes, osteoblasts and adipocytes. After 48 hours, the proliferation of HCT-116 cells cultured in CM from GM MSCs was significantly lower ($78.97 \pm 9.23\%$) compared to cells cultured in CM from native MSCs ($99.71 \pm 4.38\%$) and in fresh medium ($100.00 \pm 4.36\%$).

Conclusions: The antitumor activity of the obtained canine MSCs will be analyzed in further studies in large breed dogs with spontaneous cancer *in vivo*. This study was supported by Russian Foundation for Basic Research grant 18-44-160024 and Program of Competitive Growth of KFU.

54ASM-0103 | Proteome analysis of mesenchymal stem cells derived microvesicles

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Background: Microvesicles are spherical microstructures surrounded by a cytoplasmic membrane and containing biologically active molecules. Recent findings demonstrated that mesenchymal stem cells derived microvesicles (MVs) stimulate proliferation, migration, viability of cell, angiogenesis and regeneration. The MSCs-derived MVs are promising instrument of regenerative medicine. The aims of our work were to increase the yield of MVs using cytochalasin B and characterize the proteome and immunophenotype of cytochalsin B-induced microvesicles.

Materials and Methods: Human mesenchymal stem cells (MSCs) were used for the production of cytochalsin B-induced microvesicles (CIMVs). Immunostaining with subsequent flow cytometry analysis were used to characterize the immunophenotype of CIMV. Proteome analysis was conducted using liquid chromatography mass spectrometry method (LC-MS / MS).

Results: Proteome analysis identified 373 proteins in human MSCs and 362 proteins in CIMVs-MSCs lysates. The majority (252 molecules) of proteins were similar between MSCs and CIMVs-MSCs while 121 and 110 proteins were unique in MSC and CIMVs-MSCs, respectively. The unique proteins in CIMVs-MSCs included proteins associated with peroxisome (0.9%), lysosome (1.8%), mitochondria (6.5%), cytoplasm/nucleus (12%), cytoskeleton (20.4%), cell membrane (26%) and cytoplasm (32.4%). Analysis of the CIMVs-MSCs content revealed an increased proteins linked to cytoskeleton, peroxisomes, cell membrane and cytoplasm. In contrast, mitochondria and cytoplasm/nucleus proteins were decreased, while nucleus and secreted proteins were significantly depleted as compared to MSCs. MSCs surface receptors play role in cell to cell contact, immunomodulation and activation of signaling in target cells. We found that CIMVs-MSCs have the surface receptors similar to that of the parental human MSCs: $CD90^+$ (83%), $CD29^+$ (72%), $CD44^+$ (36%), $CD73^+$ (66%).

Conclusions: Our findings suggest that CIMVs "inherit" MSCs surface receptors and contain peripheral proteins and organelles of parental MSCs. We believe that human CIMVs-MSCs could be developed for cell-free therapy.

54ASM-0108 | Mesenchymal stem cells derived microvesicles convey mesenchymal phenotype to recipient cells

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Background: Microvesicles (MVs) are mediators of intercellular communication. They transfer soluble factors as well as surface receptors to recipient cells. MSCs surface molecules and signaling receptors play important role in MSCs biology. It is believed that MVs mediated transfer of MSCs surface receptors might be the mechanism of target cells reprogramming. Therefore, we sought to determine whether cytochalasin B induced microvesicles of mesenchymal stem cells (CIMVs-MSCs) could transfer the surface receptors to the recipient HEK293FT cells.
Materials and Methods: HEK293FT cells were pre-stained with DiO and incubated with DiD labeled CIMVs-MSCs for 24 hours. Expression of CD90 was chosen as an indicator of receptor transfer, as it is specific for CIMVs-MSCs and absent on HEK293FT cells. Expression of CD90 was analyzed using laser scanning confocal microscope Zeiss LSM 780 (Carl Zeiss, Germany) and flow cytometry BD FACS Aria III (BD Bioscience, USA).
Results: Confocal microscopy revealed fusion of CIMVs-MSCs and HEK293FT membranes and transfer of CD90 surface receptor to HEK293FT cells. Regions of DiD and partial staining with anti-CD90 antibody staining were found in the cytoplasmic membrane of HEK293FT treated with CIMVs-MSCs. Recipient cells (HEK293FT) treated with CIMVs acquired CD90 positive phenotype due to the internalization of CIMVs-MSCs membrane into the cytoplasmic membrane of the recipient cells. We determined that 99.14% of HEK293FT recipient cells acquired CD90+ immunophenotype.
Conclusions: Similar data were published by Ratajczak and colleagues, where stem cell-derived microvesicles reprogram target cells by delivering their content including mRNA to target cells. Acquiring of stem cells-like immunophenotype by differentiated recipient cells might be a sign of reprogramming. Moreover, we believe that MVs-mediated transfer of surface receptors might be a therapeutic approach to increase the permeability or accessibility of target cells for medicines or hormones.

54ASM-0160 | Biodistribution of mesenchymal stem cells derived microvesicles: in vivo and ex vivo analysis

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Background: Microvesicles (MVs) display excellent biodistribution in the body and some of them can even cross the blood–brain barrier. MVs have immense prospects for clinical application as a vectors for medicines delivery. Therefore, in this study we have investigated their trafficking in vivo. We have determined the bio-distribution of cytochalasin B induced microvesicles (CIMVs-MSCs) derived from murine mesenchymal stem cells in animal model following intravenous, subcutaneous and intramuscular injection.
Materials and Methods: CIMVs-MSCs were stained with vital membrane dye DiD and injected intravenously, subcutaneously or intramuscularly (50 μg). Mice were analyzed using IVIS Imaging System.
Results: We found that 1 hour after intravenous injection of CIMVs-MSCs the fluorescence signal was localized in internal organs presumably in lung. To accurately conclude from which organ the fluorescent signal originated the organs were imaged ex vivo. CIMVs-MSCs accumulated mainly in liver and lung, a low signal of CIMVs-MSCs was observed also in spleen and brain. At 48 hours, the fluorescent signal remained in liver and lung and started to increase in brain, heart, spleen and kidneys. These findings may be explained by an uptake of remaining in blood CIMVs-MSCs and their gradual renal excretion. Subcutaneously and intramuscularly injected CIMVs-MSCs could be detected in the injection site up to 14 days, most likely due to incorporation into the tissue and long half-life of the dye.
Conclusions: We have demonstrated that CIMVs accumulated in liver, lung, brain, heart, spleen and kidneys 48 hours after the i.v. injection. We have suggested that the subcutaneous and intramuscular injection of CIMVs-MSCs is more suitable for the local therapy.

54ASM-0161 | Microvesicles of mesenchymal stem cells demonstrate immunosuppressive activity in vivo

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Background: Mesenchymal stem cells (MSCs) demonstrate immunosuppressive activity in vitro and in vivo. It has been shown that microvesicles convey biological activity of

parental MSCs. However, the low yield of natural EVs prevents their clinical use. To increase the yield of microvesicles we use cytochalasin B. The aim of our research was to determine the immunomodulatory properties of murine MSCs and cytochalasin B induced microvesicles derived from MSCs.

Materials and Methods: Immunization of mice with sheep red blood cells (SRBC), measurement of phagocytic activity of peritoneal macrophages, determination of cellularity of spleen, thymus and bone marrow were conducted.

Results: The effect of MSCs and CIMVs-MSCs on humoral and cellular immunity of mice was analyzed. We found that CIMVs and MSC reduce the level of anti-SRBC antibody and the severity of immune reaction. The antibody titer in control, saline-treated mice, pretreated with allogenic MSCs and pretreatment with CIMVs-MSCs was 6.8 $\pm$ 1.3; 4.5 $\pm$ 0.6 ($P = 0.032$); 4.0 $\pm$ 0.57 ($P = 0.019$), respectively. The effect of allogeneic MSCs and CIMV-MSCs on neutrophil activity has been studied. There was no difference between leukocyte activation in mice treated with MSCs or CIMVs-MSCs as compared to control mice, indicating that neither MSCs or CIMVs-MSCs affected neutrophil activity. Next, we determined the effect of MSCs and CIMVs-MSCs on phagocytic macrophage activity by neutral-red dye uptake. We found that the phagocytic index of macrophages from the negative control was 0.52 $\pm$ 0.14, while it was lower in cells from mice pretreated with MSCs (0.39 $\pm$ 0.07; $P = 0.23$) or CIMVs MSC (0.34 $\pm$ 0.13; $P = 0.4$).

Conclusions: According to the received data, CIMVs-MSCs demonstrate the immunosuppressive activity similar to parental MSC.

54ASM-0181 | New experimental perspectives in adipose-derived stem cells and platelet-rich plasma in tissue engineering-based treatment of chronic skin wounds

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Background: Chronic or hard-to-heal skin wounds of diverse etiology demand urgently novel approaches that intend to accelerate wound closure and induce a correct remodeling of the newly formed fibrovascular tissue. The continuous improvements in the field of both regenerative medicine and tissue engineering have allowed the design of new and more efficacious strategies for the treatment of these wounds, based on the usage of three key methodologies: stem cells, growth factors, and biomimetic scaffolds. The adipose tissue can be considered the main source of multipotent mesenchymal stem cells, especially adipose-derived stem cells (ASC), that are found in fat grafts used in many therapeutic settings. Not only ASC show an intrinsic plasticity in giving rise to different cell types but also secrete paracrine factors involved in wound healing and angiogenesis. Dermal regeneration templates (DRT) are advanced dressing that should be safe, stable, and biodegradable and provide an adequate environment for the regeneration of tissue.

Materials and Methods: Here, we present data on our own experience in the experimental use of Integra® DRT employed in conjunction with ASC to work out the healing process, together with platelet-rich plasma as a source of growth and healing factors. Integra® DRT was sprinkled with ASC in complete medium supplemented with 10% Fetal Bovine Serum (FBS-M) or stromal vascular fraction (SVF), obtained from emulsified or non-emulsified fat, in medium supplemented with 2% platelet-rich plasma (PRP-M). The presence and differentiation of cells were evaluated by standard histochemistry and immunohistochemistry.

Results: Deposition of ASCs in FBS-M caused their integration into the scaffold as early as 1 h. ASC were found as aggregates until 6-10 days without forming organised structures. When seeded onto Integra®, SVF cells in PRP-M formed aggregates at early times, which at 7 and 10 days organized into vascular-like structures, lined by CD31- and smooth alpha actin-positive cells. With non-emulsified fat, the lacunar structures did not show an organized distribution of SVF cells.

Conclusions: The combination of SVF cells obtained from emulsified fat, PRP and advanced DRT exhibit synergistic effect on the formation of vessel-like structures indicating a step forward aimed at regenerative surgery for chronic wound healing.

54ASM-0200 | New clinical protocols of employment of adipose derived stem cells (adscs) and dermal regeneration template (DRT)

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Background: Skin and soft tissue reconstruction represents one of the most debated fields of plastic surgery. The advent of regenerative medicine showed new pathways with the use of dermal regeneration templates (DRT) and lipofilling.
Materials and Methods: Nowadays, the indications for using DRT are multiple: vascular ulcers, burns, congenital malformations, post oncological, post traumatic and post surgical reconstructions. In our experience we have been using DRT in the following body areas: Head-Neck, Lower Extremities, Upper Extremities, Hand, Trunk, Abdomen. The versatility of DRT represents a good option both in emergency and in election surgery. It represents the first choice and gold standard in the treatment of Epidermolysis Bullosa (EB), in order to solve pseudosyndactylies. Both in pediatric and adults patient, DRT has allowed us to obtain a higher rate of engraftment of skin grafts. Nevertheless DRT shows some limitations, such as not always satisfying aesthetic outcomes, hypo or hyper-pigmentation, retraction. In the last 5 years we have developed a 'three steps protocol' integra- skin grafting-lipofilling with excellent results. The aim of this study was to investigate the histological and clinical modifications occurring after lipofilling in the areas previously reconstructed with DRT and an autologous thin dermal-epidermal graft. Histological and immunohistochemical analysis were performed on nine patients to compare skin before and after lipofilling in different part of the body. Pre- and post-operative examinations (POSAS, VAS scale) were carried out as well as taking clinical photographs.
Results: Our 'three steps protocol' showed many advantages. The authors detected an overall clinical and histological improvement in all cases. Data obtained from POSAS and VAS scale showed a statistically significant ($P < 0.05$) improvement concerning all variables investigated before surgery. The biopsies revealed qualitative modifications with hematoxylin-eosin and Masson trichrome stain. Immunohistochemistry with CD31 antibody also demonstrated quantitative changes with an increased number of vessels. The photographs enabled to compare the clinical situation before and after lipofilling with better aesthetic outcomes.
Conclusions: DRT is a safe and versatile technique. The rapid use and easy to apply after extensive oncological demolition or the treatment of relapsing diseases with control of resection margins and possible radicalization, are the technical strengths with aesthetic and functional limitation. Lipofilling gave good functional and aesthetic results in the areas treated with dermal substitutes and autologous thin dermal-epidermal grafts.

54ASM-0216 | Production and functional analysis of a recombinant genetic construct that simultaneously encodes β-hexosaminidase A α- and β-subunit genes

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Background: Tay-Sachs disease (TSD) is a hereditary disorder which belongs to the group of lysosomal storage diseases and is characterized by a deficiency of β-hexosaminidase A (HexA). HexA deficiency is due to various mutations in the α-subunit (HEXA) of the enzyme gene which leads to the accumulation of GM2-gangliosides inside lysosomes of predominantly nerve cells and acute neurodegeneration. To date, there is no treatment for TSD.
Materials and Methods: In this study we developed a lentiviral vector pLX303-HEXA-P2A-HEXB encoding α- and β-subunits of the HexA enzyme, HEXA and HEXB accordingly, separated by a self-cleaving (Fu)-containing p2A-peptide nucleotide sequence which ensures independent equivalent protein translation. The resulted plasmid pLX303-HEXA-P2A-HEXB was used for the transfection of HEK293T cells. Enzymatic activity was analyzed 48 hours after transfection using MUG and MUGS fluorescent substrates. The production of HexA α- and β-subunits in genetically modified cells was confirmed using Western blot analysis. Recombinant lentivirus LV-HEXA-P2A-HEXB was produced and used for genetic modification of human cord blood mononuclear cells (hCBMCs). 2×10^7 hCBMCs-HEXA-P2A-HEXB were intravenously injected in rats.
Results: Total HexA activity in transfected HEK293T cells was increased by 35%, and α-subunit activity was increased by 10 times. After the administration of hCBMCs-HEXA-P2A-HEXB in rats HexA α-subunit activity was increased in the rat plasma. The highest activity was detected on days 6–9 after the administration and was increased by 3-4 times compared to sample before administration.

Conclusions: Thus, the use of hCBMCs-HEXA-P2A-HEXB can be an effective approach to increase HexA activity in rat plasma *in vivo*. Due to the ability of hCBMCs to overcome the blood-brain barrier, the above approach may become a promising method for TSD treatment after safety assessment. This work was funded by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities and by the Russian Government Program of Competitive Growth of KFU.

54ASM-0251 | Immunomodulating properties of mesenchymal stem cells with simultaneous overexpression of TRAIL, PTEN and IFNβ1

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Background: The discovered property of mesenchymal stem cells (MSCs) to migrate toward the place of tumor formation attracts attention of researchers. Tumor tropism of MSCs is proposed to be used for targeted delivery of antitumor agents to tumor cells. Chemotherapeutic agents and bioactive molecules which can stimulate apoptosis and inhibit tumor cell proliferation can be delivered by MSCs. In this work, the immunomodulating properties of MSCs with simultaneous overexpression of tumor necrosis factor ligand superfamily member 10 (TRAIL), tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and interferon β1 (IFNβ1) were analyzed.
Materials and Methods: Mesenchymal stem cells were isolated from human adipose tissue and genetically modified using lentivirus simultaneously encoding *TRAIL*, *PTEN* and *IFNβ1* genes separated by a self-cleaving (Fu)-containing p2A-peptide nucleotide sequence. The protein expression was confirmed by qPCR and immunofluorescence assay. To evaluate the immunomodulating properties of MSCs-TRAIL-PTEN-IFN1b mononuclear cells (PBMCs) were isolated from human peripheral blood. PBMCs were co-cultured with native and genetically modified (GM) MSCs for 72 hours, after which PBMCs were stained with antibodies specific for different human immune cell populations. The stained cells were analysed using FACS Aria III flow cytometer (BD Biosciences, USA).
Results: A slight increase ($p < 0.01$) in the number of $CD3^+CD4^-CD8^+$ T-killers was found after the cultivation with GM MSCs (110.3 ± 4.2%) compared to PBMCs that were cultured with native MSCs (98.5 ± 4.2%) or separately (100.0 ± 0.2%). An increase in the number of Th2 ($p < 0.0001$), which also play an important role in the antitumor immune response, was found in the PBMC sample after cultivation with MSCs-TRAIL-PTEN-IFN1b (152.9 ± 0.9%) compared to native MSCs (116.5 ± 2.4%) and PBMCs cultured separately (100.0 ± 1.2%). It is also worth noting that there was no statistically significant increase in the number of regulatory T-cells that are associated with suppression of the antitumor immune response.
Conclusions: Thus, MSCs with simultaneous overexpression of TRAIL, PTEN and IFN1b can be used to stimulate antitumor immunity. However, further studies of the immunomodulating and antitumor activity of MSCs in animal models are required. This study was supported by the President of the Russian Federation grant MK-236.2019.4 and Program of Competitive Growth of KFU.

54ASM-0260 | Analysis of the interaction of colon carcinoma cell-derived extracellular vesicles and human T-cells in vitro

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Background: Extracellular vesicles (EVs) isolated from tumor cells are considered as a promising vector for the targeted delivery of various antitumor agents due to their ability to fuse with recipient cells through endocytosis. Tumor cell-derived EVs carry the same antigens on the surface as their parental cells so they can be a promising source of tumor antigens for presentation to immune system cells. Thus, EVs from tumor cells can be used to develop therapeutic vaccines for the treatment of cancer. The aim of the present study was to analyze the interaction of EVs derived from colon carcinoma HCT-116 cells with human T-cells in vitro.
Materials and Methods: Mononuclear cells (PBMCs) were isolated from peripheral blood by Ficoll density gradient centrifugation. Vybrant-DiO-stained PBMCs were cultured with Vybrant-DiD-stained HCT-116 cell-derived EVs (40 μg/mL) for 3 days. After that PBMCs were stained with fluorescent-labeled antibodies to CD3, CD4, CD8a, CD38, CD25, CD56, CD107a, CD127, CCR6 and CXCR3. The results were analyzed using flow cytometry. The interaction of EVs and PBMCs was analyzed using confocal microscopy.
Results: It was shown that 4% of T-cells and 32% of granulocytes were able to take up HCT-116 cell-derived EVs. This can be explained by the ability of neutrophils to phagocytosis. It was also shown that the cultivation of PBMCs with HCT-116 cell-derived EVs increases the number of HLA-DR+CD38+ activated T-killers by 12% and CD107+ T-cells by 32% compared to control cells cultured without EVs. An increase in T helper 2 (Th2) population by 7% after the cultivation with HCT-116 cell-derived EVs was also observed.

There was no statistically significant difference in the number of natural killers.

Conclusions: Thus, due to the ability of tumor cell-derived EVs to present tumor antigens to immune system and activate the antitumor immune response, they are considered as a promising tool for the development of therapeutic antitumor vaccines. However, further studies to investigate the mechanisms of immunomodulating properties of tumor cell-derived EVs are required. This work was funded by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities and by the Russian Government Program of Competitive Growth of KFU.

54ASM-0265 | Human Huntington's disease iPSC-derived striatal-like neurons display altered maturation and mitochondrial dynamics

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Background: Multiple mechanisms have been implicated in the pathogenesis of Huntington's disease (HD), a disorder caused by an expansion of CAG repeats in the *HTT* gene. Pathological processes include defects in mitochondrial and energy metabolism associated with increased levels of reactive oxygen species (ROS). Induced pluripotent stem cell (iPSC) technology and derived neural stem cells (NSC) and striatal-like neurons used for disease modelling has given new insights into disease mechanisms.

Materials and Methods: In this study, we aimed to characterize mitochondrial function, dynamics and electrophysiological changes in late-onset HD NSC and differentiated striatal-like neurons.

Results: HD-NSC exhibited a tendency for diminished maximal respiration and increased proton leak, but unchanged mitochondrial or cellular levels of ROS, when compared to control cells. Mitochondrial morphological analysis showed a lower degree of branching and reduced Feret's diameter, along with increased number of mitochondria, indicative of enhanced mitochondrial fragmentation. iPSC derived striatal like neurons expressed selective identity, at day 45, confirmed by the expression of striatal GABAergic markers, namely GAD65/67 and DARPP-32. At day 60, mature neuronal cells were confirmed through increased $MAP2^+$/$bIII\text{-}tubulin^+$ ratio. Moreover, a tendency for an increase in the yield of $DARPP\text{-}32^+$ neurons was observed as neurons became more mature, mostly in control cells. At day 80, full maturation stage was reached, as most cells were positive for SMI-32 neuronal marker. Whole-cell patch-clamp recordings showed that HD neurons displayed a lower frequency and amplitude of mIPSC and a lower GABA receptor-mediated current density in comparison with control neurons. In differentiated neurons, the majority of mitochondria were shown to be paused; from those that were mobile, mitochondrial mean velocity was reduced in HD *versus* control neurons.

Conclusions: In conclusion, HD-NSC presented altered mitochondrial function and morphology and HD-iPSC derived striatal neurons further displayed impairment of neuronal activity and reduced mitochondrial morphology and movement.

54ASM-0282 | Unveiling the effects of hgps using human induced pluripotent stem as a model

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Background: Hutchinson-Gilford Progeria Syndrome (HGPS) results from a point mutation in one allele of the *LMNA* gene, causing the formation of a farnesylated form of Lamin A – Progerin – that intercalates and accumulates in the nuclear envelope, leading to cellular changes that culminate in premature ageing.

The cardiovascular system is greatly impaired in HGPS patients, causing death usually by either myocardial infarction or stroke, at an average age of 14 years-old. Although HGPS has been widely studied in smooth muscle cells, little is known about its effects on cardiac cells.

We aimed to assess the cellular and molecular changes that occur in human HGPS cardiomyocytes.

Materials and Methods: Human induced pluripotent stem cells (hiPSCs) derived from HGPS patients were differentiated into cardiomyocytes through modulation of the Wnt signaling pathway.

Control cell lines containing the same genetic background as the HGPS-hiPSCs were created by CRISPR/Cas9: one containing a frameshift mutation Progerin knockout, and other a complete correction of the point mutation.

HGPS-hiPSC cardiomyocytes were collected following 15 and 20 days of differentiation and characterised by immunocytochemistry and flow cytometry.

Results: Progeria hiPSCs were successfully differentiated into cardiomyocytes. The onset of beating was identical in HGPS-hiPSC cardiomyocytes, genome-edited control cell lines, and wild-type embryonic stem cells (i.e. day 6 of differentiation). Nevertheless, the percentage of cardiomyocytes obtained from HGPS hiPSCs was lower than the controls. Additionally, HGPS hiPSC-derived cardiomyocytes exhibited a lower beating rate than the control counterparts, and the resulting cardiac fiber bundles appeared less robust. Immunostaining showed that at day 20 of differentiation progerin accumulation is detectable in HGPS iPSC-derived cardiomyocytes, with most cells expressing low levels of Progerin.

Conclusions: The presence of the HGPS LMNA mutated allele showed to cause deleterious effects not only on the beating rate and robustness of the produced cardiac tissue, but also on the efficiency of the cardiac differentiation itself.

54ASM-0295 | The use of chitosan - silk fibroin - graphene oxide hydrogels for the regeneration of deep-layer skin wounds

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Background: Full-layer skin wounds are an urgent problem of medicine. The development and application of hydrogels are a promising approach for their treatment.

Materials and Methods: Hydrogels D (Daba silk fibroin + chitosan) and D + G (Daba silk fibroin + chitosan + graphene oxide) were developed. Hydrogels were tested for cytotoxicity on skin fibroblasts using MTS test. Hydrogels were applied on modeled full-layer round skin defect with a diameter of 1 on the scapular part of the back Wistar rats. The tissues examined 7 and 14 days after wound formation using histology and immunohistochemistry.

Results: D and D + G hydrogels supported the proliferation of skin fibroblasts for at least 96 hours. In group D + G cells had normal fibroblast-like morphology, in D group a significant part of the cells was detached.

7 days after application of D and D + G on skin wound was represented by a fibrous connective tissue of light blue colour in Heidenhain azocarmine staining, richly vascularized and richly infiltrated with leukocytes, erythrostasis and erythrocyte diapedesis were observed. Only marginal epithelization was observed. After 14 days of application of the hydrogel D, the epithelization of the wound was still only marginal, the collagen of the underlying connective tissue was light blue, rich vascularization and erythrostasis persisted. However, the use of hydrogel D + G for 14 days showed complete epithelization of the wound, the underlying connective tissue contained glands and hair follicles, the collagen was intensely blue.

Conclusions: The use of hydrogels based on a combination of chitosan, silk fibroin and graphene oxide has shown effectiveness in the regeneration of deep skin wounds.

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54ASM-0298 | Transcriptome sequencing gene engineering of human umbilical cord blood mononuclear cells

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Background: Hematopoietic stem cell (HSC) gene therapy is becoming a promising and powerful universal strategy for the treatment of various human diseases. Umbilical cord blood considered the be one of the most attractive and available sources for HSC suitable for *ex vivo* genetic modification and transplantation. Transplanted genetically modified umbilical cord blood mononuclear cells (UCB-MCs) are capable of expression of the therapeutic transgene, which eliminates pathological disorders. At the same time, little is known about the individual functional differences between isolated UCB-MCs at the transcriptome level and the effect of genetic modification on its change. In the present study, we determined the impact of modification UCB-MS with adenovirus Ad5-EGFP on expression recombinant protein and RNA profile of control and modified cells.

Materials and Methods: Total mRNA from genetically modified and non-treated cells were sequenced on the Illumina NextSeq 500 platform in 2*75 bp mode. After quality control, reads were aligned to human reference transcriptome GRCh38 using Kallisto pseudoaligner. Differentially expressed transcripts and genes were calculated with R package 'sleuth'.

Results: It has been shown that modified UCB-MCs expressed recombinant protein - EGFP. We comprehensively profiled the whole-transcriptome landscape of human UCB-MC. Transcriptomic analysis reveals 2.4-2.8*106 paired-end reads. We also demonstrated that genetic modification does not lead to global change in the transcriptome profile. Only

EGFP (log2FC=7.15, $q < 0.05$) gene demonstrate significantly increased transcription after gene modification. The primordial analysis showed that UCB-MC expressed various cytokines, chemokines, and growth factors.

Conclusions: Our pilot study showed that genetic modification of UCB-MC with adenoviruses does not affect the transcriptome profile of the cells. At the same time, genetic modification with recombinant adenovirus ensures the ability of cells for synthesis of recombinant proteins. The use of UCB-MC as a vehicle for delivery of therapeutic genes can be used as a platform for creating gene-cell preparations which meet the criteria of biological safety and efficiency. Thus, transcriptional profiling is essential when creating personalized gene-cell products. This work was supported by the RFBR grant № 18-44-160029.

54ASM-0311 | Lentiviral-based reporter constructs for bioluminescent in vivo imaging

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Background: The tumor microenvironment is a dynamic environment that consists of different cells, including mesenchymal stem cells (MSCs). Animal models of tumor xenografts are important for the study of carcinogenesis in vivo. However, the lack of immune system in immunodeficient mice has limitation in informational content. Cells of the immune system and MSCs are important for tumor growth and formation. The aim of the present study was to create a xenograft tumor model by subcutaneous injection of genetically modified human neuroblastoma cells (SH-SY5Y) expressing the bioluminescent luciferase protein (SH-SY5Y-ffLuc) to immunocompetent mice.

Materials and Methods: SH-SY5Y cells were genetically modified using recombinant lentivirus LV-ffLuc. SH-SY5Y-ffLuc cells were cultured with selective antibiotic puromycin (Sigma-Aldrich, USA) at a concentration of 1 μg/mL to obtain a stable cell line. The relative intensity and stability of the luciferase signal in SH-SY5Y-ffLuc were analyzed by ONE-Glo™ Luciferase Assay System. A xenograft model was obtained by subcutaneous injection of 300 μl SH-SY5Y-ffLuc cells in a Matrigel Matrix suspension into 6-week-old C57BL/6 mice. For in vivo imaging, mice were injected with D-luciferin (150 mg/kg), which was dissolved in phosphate-buffered saline. Images were obtained using IVIS-Spectrum imager (Caliper Life Sciences, USA).

Results: After adding ONE-Glo Luciferase Assay System to SH-SY5Y-ffLuc cells, the maximum luminescent signal intensity was detected after 10 minutes and maintained over 30 minutes. The kinetics of the bioluminescence signal intensity after intraperitoneal D-luciferin injection showed that the SH-SY5Y-ffLuc cells formed a tumor. Thus, subcutaneous xenograft of human neuroblastoma tumor cells to immunocompetent mice is successful.

Conclusions: The obtained tumor model will be used for further studies of carcinogenesis and determine the effect of MSCs on tumor growth. This work was funded by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities and by the Russian Government Program of Competitive Growth of KFU.

54ASM-0380 | Cognitive functions of mice with the model of the first type of diabetes mellitus

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Background: Diabetes mellitus (DM) is an endocrine disease characterized an increase in blood glucose levels. Central nervous system is one of the targets of hyperglycemia. One of the pathological mechanisms of the effect of the hyperglycemia is a violation of the antioxidant system. The aim of our work was to study the cognitive functions and the level of oxidative stress in brain of mice with type 1 DM.

Materials and Methods: Experimental DM was caused by alloxan (200 mg/kg, i.p) which increased the blood glucose concentration more than 10 mM. Behavioral experiments were performed after 45 days of DM. Morphology of the pancreas showed a decrease in the number of insulin-positive cells in the islets of DM mice. The cognitive functions were assessed by Novel Object Preference, T-maze and Morris water maze. The level of oxidative stress in brain tissues was evaluated by the concentration of malondialdehyde (MDA) and activity of glutathione peroxidases (GPs).

Results: The Novel Object Preference is test for recognition memory. In this test the ratio of the time that animal spends to study a new object to the time of study of an old object was estimated. In mice with DM this ratio was lower compared to the control group. The T-maze allows exploring the working memory where the selection of the new sleeve was assessed after 2 trials. Mice with DM did not demonstrate the alteration of working memory. In the Morris Water Maze, the percentage of trained DM mice was lower compared to the control group and DM mice spent more time to find the hidden platform. In one day after training session mice with DM spend more time to get the platform. In animals with DM the concentration of MDA in brain homogenates was higher and the activity of GPs was lower compared to the control group.

Conclusions: Our study indicates that hyperglycemia induces the decreased of working memory and learning ability

which may partially mediated by the oxidative stress in brain tissues which impairs neuronal functions.

54ASM-0382 | Development of seizure-like events in hippocampal neurons of rats with prenatal hyperhomocysteinemia

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Background: Homocysteine is a metabolite of the indispensable amino acid, methionine, and occupies a position in the methionine cycle at the intersection between S-adenosylmethionine, vitamin B12 and folic acid. High levels of homocysteine (hyperhomocysteinemia, HHCy) signify a breakdown in the cycle that has implications in many disease processes, including cognitive impairment and neurodegenerative diseases. It was shown, that HHCy can contribute to seizures in patients with Down syndrome, depression and in alcohol withdrawal. Abnormal accumulation of homocysteine during pregnancy induces learning deficits in offspring at early postnatal development. It was shown that during the development of brain chronic HHCy increases the excitability of nervous circuits and may provoke seizures. In this study we investigated excitability of hippocampal neuronal networks from rats with prenatal HHCy.
Materials and Methods: Experiments were performed on hippocampal slices of Wistar rats during second weeks after birth. Pups with prenatal HHCy were born from females received daily methionine with food 2 weeks before and during pregnancy and 2 weeks after delivery. Electrical activity was recorded using extracellular field electrodes in the CA3 pyramidal cell layer of hippocampus. Seizure-like events (SLE) were evoked by high KCl containing extracellular solution.
Results: In control group application of 6 mM KCl increased of the frequency of multiunit activity of hippocampal neurons. Elevation of KCl concentration up to 8.5 mM induced SLE in 62% cases (n = 32) after 4.1 ± 0.5 minutes of perfusion. In rats with prenatal HHCy the increase of background neuronal firing was observed at KCl concentrations from 3.5 to 6 mM with generation of epileptiform activity in 47% of the slices. Further elevation of the extracellular potassium to 8.5 mM evoked epileptiform activity in 72% of slices with shorter latency of seizure onset (0.9 ± 0.2 min, n = 15). The spectral analysis of SLE did not reveal significant changes of power of epileptiform activity.
Conclusions: Our data suggest that hippocampal neurons of immature rats have higher sensitivity to depolarizing agent which may underlie a high risk of seizure appearance in postnatal life in case of maternal HHCY. This work was supported by RFBR № 18-015-00423 and by the subsidy allocated to Kazan Federal University for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

54ASM-0383 | Learning ability and spatial memory in adult rats with prenatal hyperhomocysteinemia

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Background: Elevated level of homocysteine during pregnancy is resulted in preeclampsia, fetus pathologies, growth restriction due to endothelial dysfunctions of placenta and oxidative stress. In our previous study we demonstrated an early postnatal developmental impairments, including delay in reflexes maturation, motor and cognitive dysfunctions of the offspring with prenatal hyperhomocysteinemia (hHCY). The aim of the present work was to analyses the changes of cognitive functions of adult rats with prenatal hHCY.
Materials and Methods: The experiments were carried out on Wistar rats (P 72-90) divided into 2 groups in accordance with the diet of females: 1) the control group (n = 25); 2) the group Hcy (n = 25) received daily methionine (7.7 g/kg body weight) with food, before pregnancy and 2 weeks after delivery. The level of homocysteine was 27 ± 2 μM (n = 15) in female. Elevated level of Hcy was observed in their offspring in late postnatal life in 76% of animals (19.5 ± 1.5 μM, n = 25, P 72-90). We analyzed behavior in the Open field, Morris water maze and T maze tests.
Results: Total locomotor activity in Open Field test was significantly higher in the Hcy group compared to control. In the T-maze selection of the new sleeve after 2 trials were considered as positive results. In the control group 69 ± 4% of animals chose the new sleeve for investigation and in the Hcy group – only 36 ± 4%. In the Morris water maze only 87% of rats from the Hcy group learned to find the hidden platform (95% in control). During the retention trial session in 1 day after learning rats from the hHCY group spend more time to find the platform.
Conclusions: It was concluded that high homocysteine level during pregnancy decreased ability to learn and induces spatial memory impairment in adult life. This work was supported by Russian Science Foundation № 20-15-00100 and the subsidy allocated to Kazan Federal University for the state assignment № 0671-2020-0059 in the sphere of scientific activities.

S12 – NEW TRENDS IN THYROID RESEARCH AND SURGERY

54ASM-0097 | Comparison of hepatoprotective and proliferation stimulating effects of Xymedon, L-ascorbic acid and Xymedon conjugate with L-ascorbic acid on the model of CCl4-modulated hepatitis in rats

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Background: At present, many factors (unfavorable environment, poor nutrition, drug metabolites, etc.) affect on the state of the liver and can cause its diseases, so studying the properties of potential hepatoprotectors is an important task. Xymedon ((N-β-hydroxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxopyrimidine) (I) has hepatoprotective properties previously found, that were enhanced in its conjugate with L-ascorbic acid (II), and therefore, it is important to comparison the effectiveness of conjugate (II) and the original substances in equimolar doses.

The purpose of this study was to compare the hepatoprotective effect of Xymedon, L-ascorbic acid (III) and Xymedon conjugate with L-ascorbic acid and to study their effect on the proliferative activity of liver tissue.

Materials and Methods: The liver damage was exposure by carbon tetrachloride in Sprague Dawley rats, which after that were treated with preparations (I), (II), (III) in doses of 1.45 μM/kg (0.24, 0.50, 0.26 mg/kg, respectively) during 5 days. Then animals were euthanized to examine the liver morphology. 2 hours before euthanasia bromodeoxyuridine (BrdU) was administered intraperitoneally at a dose of 50 mg/kg to study the liver tissue proliferative activity, and then frozen liver sections were immunohistochemically stained. The analysis was undertaken on the LSM 780 confocal microscope (Carl Zeiss).

Results: As a result, a more pronounced decrease of damaged liver tissue areas was revealed with the administration of (II) compared with (I) and (III): 72.2 ± 4.7% in control, 27.3 ± 4.2; 34.3 ± 2.9 and 48.7 ± 4.1% in groups with administration of (II), (I) and (III), respectively. Immunohistochemical studies showed that the proliferative activity index of liver cells was significantly higher with the administration of (I) and (II) (1.9 ± 0.4% and 2.2 ± 0.4%, respectively) than with the administration of (III) (0.9 ± 0.3%), in the control and reference (intact) animals (1.0 ± 0.3% and 0.9 ± 0.3%, respectively).

Conclusions: The study showed that the conjugate of Xymedon with L-ascorbic acid has more pronounced hepatoprotective properties and liver tissue proliferative activity compared to the original substances.

54ASM-0131 | Immunomodulatory properties of functional food additives on the base of pectin and complexes of pectin with Ca, Cu and Zn

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Background: Pectin polysaccharides are known detoxicants and exhibit a number of beneficial properties for the body. Pectin and its complexes with macro- and microelements can be used as prophylactic functional food additives. The aim was to study immunomodulatory properties of pectin and its complexes with Ca, Cu, Zn.

Materials and Methods: The study was performed on male Wistar rats weighing 180-200 g at the age of 2-2.5 months. Against the background of pectin administration (group 2) and pectin complexes with Zn (group 3), Cu (group 4) and Ca (group 5), immunodeficiency was induced in animals by intraperitoneal administration of cyclophosphamide at a dose of 25 mg/kg. Group 1 (control) was administered with water. The number of leukocytes (WBC), lymphocytes (LYM, LYM%), monocytes (MON, MON%), granulocytes (GRA, GRA%) were studied in dynamics on the automatic Mythic 18Vet hematology analyzer.

Results: WBC decreased by 41.3% under the influence of cyclophosphamide in control. The number of all leukocytes subpopulations decreased approximately by half, the percentage ratio of LYM%, MON%, GRA% remained. In experimental groups, the decrease in WBC was by 34.1, 26.4, 22.2, and 32.2% in groups 2, 3, 4, 5, respectively. In contrast to the control, the number of monocytes and granulocytes, i.e. phagocytes, decreased less in experimental groups. On the 8th day after cyclophosphamide injection, the most complete recovery of WBC was observed in groups 3 and 4. On the 15th day, the full recovery of total number of leukocytes did not occur in groups 1, 2 and 5. In third group, WBC exceeded the initial values by 36.8%, and in the 4th group, WBC did not differ from the initial level.

The study was funded by RFBR, project № 18-013-01177.

Conclusions: Pectin and pectin complexes with Cu, Zn, Ca exhibit immunomodulatory properties; the greatest stimulating effect was found for pectin complexes with zinc and copper.

54ASM-0167 | Portal blood flow is impaired in obese subjects—The role of 13c methacetin breath test

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Background: Liver function is highly impaired in patients with chronic liver diseases. It can be assessed by dynamic testing of certain biochemical compounds. Furthermore, liver biopsy brings future complications, despite being considered the 'gold standard' for studying hepatic damage and does not provide functional information. We aimed to test the dynamic function of 13C methacetin in healthy subjects and in patients with non-alcoholic fatty liver disease (NAFLD).

Materials and Methods: In 81 subjects (age: 44.8 ± 1.6 years, body mass index: 28.5 ± 0.7 kg/m^2, female: 44%, NAFLD: 67%, obese: 33%), portal blood flow (delta over baseline value after 15 min: DOB15) and microsomal function (cumulative per cent dose recovery after 30 min: cPDR30) as representative markers of liver function, were assessed by orally administered 13C methacetin. Subjects were stratified according to body mass index (BMI) and presence of liver steatosis (NAFLD). Liver steatosis was assessed and graded by ultrasonography (0-3).

Results: Mean degree of liver steatosis in NAFLD subjects was 1.4 ± 0.1 (mean ± SEM). Post-hoc analysis revealed portal blood flow to be more impaired in obese when compared to lean (12.7 ± 1.1 vs. 19.1 ± 1.6 ‰, $P = 0.002$) than in NAFLD when compared to healthy subjects (14.5 ± 1.0 vs. 18.0 ± 1.4 ‰, $P = 0.008$). The prevalence of abnormal portal blood flow (DOB15 < 14.5 ‰) was also higher in obese when compared to lean (63 vs. 23%, $P = 0.005$) than in NAFLD when compared to healthy subjects (56 vs. 30%, $P = 0.035$). Microsomal function was preserved, irrespective of BMI or presence of liver steatosis.

Conclusions: Increased adiposity was associated with a major impairment of hepatic blood flow as assessed by dynamic test of liver function, rather than liver steatosis alone. Further evidence needs to be gathered concerning a potential link between different markers of hepatic damage and liver function parameters, whether assessed biochemically or dynamically.

54ASM-0168 | Physical inactivity predisposes to a higher risk of subclinical atherosclerosis

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Background: Non-alcoholic fatty liver disease (NAFLD) is a worrisome chronic liver disease affecting almost 25% of the worldwide population. Physical inactivity, as a marker of sedentary lifestyles, is an independent risk factor for atherosclerosis. We studied the link between carotid intima-media thickness (cIMT), epicardial thickness (ET), liver steatosis and physical activity levels, in a cohort from Southern Italy.

Materials and Methods: Ninety-eight subjects (age: 43.6 ± 1.5 years, body mass index: 28.6 ± 0.7 kg/m^2, female: 44%, NAFLD: 37%, obese: 40%) were non-invasively studied for cIMT (mm, abnormal ≥ 1 mm), ET (mm, abnormal ≥ 5 mm) and liver steatosis (degree: 0-3) by ultrasound (Noblus® Hitachi, 3.5 and 7.5 MHz probes, Italy). Weekly physical activity was assessed by the International Physical Activity Questionnaire, and later transformed to 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 physical activity levels (sedentary, slightly and moderately active), sedentary subjects reported significantly higher cIMT and liver steatosis as compared to slightly and moderately active subjects (cIMT: 0.9 ± 0.03 vs. 0.7 ± 0.05 and 0.8 ± 0.03 mm, $P = 0.005$ and $P = 0.041$, respectively; degree of liver steatosis: 1.5 ± 0.1 vs. 0.6 ± 0.2 and 0.7 ± 0.1, $P = 0.001$ and $P < 0.0001$, respectively). Sedentary subjects also reported higher degree of ET as compared to moderately active subjects (7.8 ± 0.4 vs. 6.1 ± 0.4 mm, $P = 0.005$). Furthermore, the prevalence of abnormal cIMT was significantly higher in sedentary subjects when compared to slightly active subjects ($P = 0.024$), and of abnormal ET when compared to both slightly and moderately active subjects ($P = 0.044$ and $P = 0.007$, respectively).

Conclusions: In our clinical setting, physical inactivity is associated with increasing fat deposition as markers of subclinical atherosclerosis. Over 40% of our cohort did not meet the minimum physical activity levels, stressing the need for more encouraging advice from physicians and healthcare personnel.

54ASM-0169 | A researcher approach to clinical practice – 'Foie Gras' three years later

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Clinical practice deserves a lot of attention from researchers nowadays. Researchers force healthcare personnel to advance their current medical knowledge and to continuously search for new ideas, to ameliorate the management of patients. Research should therefore be encouraged and nurtured, in order to enhance the adequacy and the efficacy of the interaction between patients and physicians. Communication skills are crucial, but psychology and knowledge of the major health determinants in each patient also plays an important role.

Research is hence a way to complete and adequate our current knowledge based on pre-existing information and health conditions, but also a useful tool to act on socio-economic inequalities worldwide. By acquiring a more comprehensive picture of health determinants (including environmental, cultural and socio-economic factors), we will be able to better manage patients and their behaviour, to perform a correct diagnosis and, eventually, a sustainable treatment, but also, of note, to propose measures and policies for primary prevention. Research should consider a more realistic idea of our healthy environment, in order to verify our hypotheses and to adequately assess their actual role.

There are several reasons why research is and will always be demanded in clinical practice: patients and doctors need to understand benefits, risks, costs and sustainability of treatments; the answers to specific questions must be applicable to a broad category of subjects; we need simple but comprehensive databases to answer every possible intricacy and interplay within scientific data; the management of a disease or health condition is often precluded by the judgement of doctors, which can be based on health costs and/or what is more important and relevant to them, forgetting about the living environment, social, familiar or economic condition of each patient, and irrespective of the real sustainability of the proposed approach.

Researchers should put into perspective the real needs of people with respect to their health (including the crucial aim to maintain health, and not only to treat diseases) and, first and foremost, with respect to human condition.

54ASM-0208 | Being moderately active is linked to less fat predisposition—Perspectives from the 'foie gras' project

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Background: Non-alcoholic fatty liver disease (NAFLD) is increasingly becoming one of the most common chronic liver diseases due to, but not only, an also increasing prevalence of obesity. Healthy lifestyles (i.e. being physically active and consuming a generally hypo-caloric diet, among others), are currently the best preventive measurements and therapy to decrease its incidence and stop its evolution. We aimed to study the link between increased fat predisposition and being physically active in our clinical setting located in Apulia.

Materials and Methods: In 98 subjects (age: 43.6 ± 1.5 years, body mass index: 28.6 ± 0.7 kg/m^2, female: 44%, NAFLD: 63%, obese: 40%), physical activity was assessed by the International Physical Activity Questionnaire (Minetto et al. 2018) on a weekly basis, and transformed to Metabolic Equivalent Tasks (METs; 1 MET=3.5 mL/Kg/min of oxygen consumption or 1.5 Kcal/Kg/hr). Waist circumference (cm) was measured following the indications of the Revised National Cholesterol Education Programme-Adult Treatment Panel III (R-ATPIII) and the International Diabetes Federation (IDF). Visceral fat thickness (mm) was measured by ultrasound (Noblus® Hitachi, 7.5 MHz probe, Italy). Liver steatosis was assessed and graded by ultrasonography (0-3).

Results: Mean degree of liver steatosis in NAFLD subjects was 1.6 ± 0.1 (mean ± SEM). When classifying subjects according to physical activity (sedentary < 1000 METs/week, slightly active: 1000-2000 METs/week and moderately active > 2000 METs/week), sedentary subjects had higher waist circumference as compared to slightly and moderately active subjects (R-ATPIII: 106.2 ± 2.7 vs. 93.9 ± 4.3 and 93.9 ± 2.5 cm, $P = 0.045$ and $P = 0.003$, respectively; IDF: 99.9 ± 2.2 vs. 85.8 ± 3.5 and 88.1 ± 2.1 cm, $P = 0.002$ and $P < 0.001$, respectively). Visceral fat thickness was furthermore increased in sedentary when compared to slightly and moderately subjects (61.5 ± 3.8 vs. 42.3 ± 6.2 and 42.9 ± 3.7 mm, $P = 0.038$ and $P = 0.002$, respectively). Degree of liver steatosis, as an additional marker of liver damage in metabolic syndrome, was also significantly higher in sedentary when compared to slightly and moderately active subjects (1.5 ± 0.1 vs. 0.6 ± 0.2 and 0.7 ± 0.1, $P = 0.001$ and $P < 0.0001$, respectively). Sedentary subjects were also heavier when compared to slightly and moderately active

subjects (31.9 ± 1.0 vs. 26.2 ± 1.6 and 26.5 ± 0.9 kg/m^2, $P = 0.007$ and $P < 0.001$, respectively).

Conclusions: In our clinical setting from Southern Italy, almost 40% of our population did not meet the minimal physical activity levels (≈1000 METs/week, Piercy et al. 2018), of which 85% had NAFLD and 62% were obese. This trend occurs in a fatty background which predisposes the individual to several cardiovascular risks, and shapes the worries and needs of future healthcare in the Mediterranean area.

54ASM-0336 | The cytoprotective effect of Xymedon and its conjugate with L-ascorbic acid on the cell line of normal human hepatocytes Chang Liver

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Background: Several studies have shown that the drug Xymedon have reparative and regenerative action. Also shown, that its conjugates with biogenic acids contribute to the regeneration of the liver tissue after CCl4-induced damages. The aim of this work was to study the cytoprotective effect of the conjugate of Xymedon with L-ascorbic acid (substance II) in comparison with Xymedon (1-(beta-hydro xyethyl)-4,6-dimethyl-1,2-dihydro-2-oxopyrimidine) (substance I).

Materials and Methods: The experiments were performed on the Chang Liver cell line, obtained from the Russian collection of cell cultures of D.I. Ivanovskii Institute of Virology (Moscow). The cytoprotective effect was studied on cells with d-galactosamine-induced (at a concentration of 150 and 80 mM) damages. Cells were stained with HOECHST and Propidium iodide. The analysis was performed on a Cytell Cell Imaging System analyzer. Also, the ultrastructure of cells was investigated on a Hitachi HT7700 Exalens electron microscope.

Results: Substances I and II have shown a cytoprotective effect at a minimum concentration of 35 μM: the number of viable cells was 32.8% ± 1.56% and 31.0% ± 6.6%, respectively, vs control (150 mM d-galactosamine) 1.1% ± 0.4%. The concentrations of substances I and II (125 and 250 μM, respectively) were determined at which the maximum number of viable cells was observed (50.9% ± 1.6 and 52.7% ± 4.4%, respectively).

On microphotos, which was obtained using electron microscopic, revealed the influence of substances I and II led to a decrease in signs of damage to nuclei. Also, influence of substances I and II, led to increase in the nuclear-cytoplasmic index compared with control, which is 34.13% ± 3.91%, 38.84% ± 4.93%, respectively, and in the control (80 mM d-galactosamine) 28, 65% ± 3.28%.

Conclusions: In the result of the study on the Chang Liver cell line, the cytoprotective effect of the drug Xymedon (1-(beta-hydroxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxopyrimidi ne) and its conjugate with L-ascorbic acid was detected and the effective concentrations of substances were established.

54ASM-0372 | MBOAT7 rs641738 variant is linked to altered portal blood flow: results of dynamic assessment of liver function using 13c methacetin test in 92 subjects

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Background: *MBOAT7* rs641738 is a common genetic variant associated with non-alcoholic fatty liver disease (NAFLD), inflammation and liver fibrosis, which increases the progression from simple liver steatosis to hepatocellular carcinoma. Liver biopsy is still considered the 'gold standard' method in quantifying the degree of liver injury in patients with NAFLD, despite potential complications due to its invasive nature. Consequently, non-invasive tools are seen as alternatives to liver biopsy. Here, we aimed to study the dynamic function of the liver by oral administration of ^{13}C methacetin in individuals carrying the *MBOAT7* rs641738 variant.

Materials and Methods: In 92 subjects (age: 43.9 ± 1.5 years, body mass index: 28.8 ± 0.7 kg/m^2, females: 42%, NAFLD: 63%, obese: 40%), portal blood flow (delta over baseline value after 15 min: DOB15) and microsomal function (cumulative per cent dose recovery after 30 min: cPDR30) were assessed by oral administration of ^{13}C methacetin. Subjects were stratified according to allele types (wild-type, heterozygous, mutant). Liver steatosis and fibrosis were assessed non-invasively by ultrasonography (degree: 0-3) and acoustic radiation force impulse (ARFI) technique (degree: 0-4), respectively.

Results: Mean degree of liver steatosis and liver fibrosis in NAFLD patients was 1.6 ± 0.1 and 1.1 ± 0.2 (mean ± SEM), respectively. *MBOAT7* genotypes were neither associated with liver fibrosis nor steatosis. Portal blood flow was significantly lower in homozygous carriers of the *MBOAT7*

risk variant as compared to heterozygous subjects (DOB15: 11.1 ± 1.5 vs. 16.4 ± 1.0 ‰, $P = 0.047$), even falling below the cut-off value (DOB15 < 14.5 ‰). Microsomal function tended to be lower in subjects who are homozygous for the *MBOAT7* risk allele for in comparison to heterozygous subjects (9.9 ± 0.9 vs.12.4 ± 0.6 ‰, $P = 0.065$), but remained well above the cut-off value (cPDR30 < 8.1%).

Conclusions: The *MBOAT7* rs641738 variant might be associated with impaired portal blood flow. Microsomal function was preserved but tended to decrease in carriers of the gene variant, too. In the future, more studies should link the *MBOAT7* rs641738 variant with NAFLD severity using non-invasive liver function tests.

S13 – IRON & AGING

54ASM-0126 | The neuromuscular apparatus of the calf muscles of the rat with restriction of motor function

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Background: Restriction of motor function as a result of the development of pathological processes or changes in motility conditions, for example, under the action of weightlessness during space expeditions, causes significant transformations in all structures of motor systems. The speed and intensity of the transformations can be dependent on morphological and functional features of neuromuscular apparatus.

Materials and Methods: In this study, we evaluated the effect of simulated gravitational unloading (7 days) on the electromyographic parameters of slow m. soleus, mixed m. gastrocnemius and fast m. tibialis anterior. The experiments were carried out on laboratory rats weighing 180-200 g with all bioethical norms. During sciatic nerve stimulation, M-wave caused by activation of efferents and H-reflex caused by activation of motor neurons through afferents Ia was recorded. The H/M ratio was also calculated and the M-wave decrement test was performed. The control group was a group of intact animals.

Results: It is shown that gravitational unloading causes an increase in the reflex excitability of motor neurons of the spinal motor center m. soleus and m. gastrocnemius (the H-reflex threshold was reduced; the maximum amplitude of the H-reflex and H/M ratio was increased). Under experimental conditions, the activity of peripheral structures of the neuromuscular apparatus m. gastrocnemius increases (M-wave threshold has been reduced). The reliability of neuromuscular synaptic transmission is impaired in m. soleus and m. gastrocnemius (M-wave decrement was significantly increased). Electromyographic characteristics m. tibialis anterior did not changed.

Conclusions: Thus, the short-term gravitational unloading causes changes in the functional state of the neuromuscular apparatus of anti-gravity muscles and does not affect on the neuromuscular apparatus in the fast m. tibialis anterior. It is assumed that the cause of hypogravitational effects is the limitation of peripheral afferent signaling, mainly from support receptors. The reported study was funded by RFBR according to the research project No. 19-04-01067.

54ASM-0127 | Electrical activity of lower limb muscles in patients with coxarthrosis

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Background: We studied electrical activity (EA) in 20 healthy test volunteers and 27 patients with right and left coxarthrosis with their consent. EA was recorded in the rectum, medial and lateral heads of the quadriceps femoris muscle (qGM), and in the soleus, medial and lateral gastrocnemius triceps surae muscle (TSM).

Materials and Methods: The background EA was recorded at rest, and then with maximum muscle contraction. The mean amplitude of EA was determined.

Results: In healthy subjects and patients with coxarthrosis, background EA in the muscles of the thigh and lower leg is absent. In patients with both left- and right-sided coxarthrosis, a significant decrease in the amplitude of randomly induced EA of qGM was noted, which was least expressed in its medial head. The amplitude of randomly induced EA in different TSM heads in patients was also reduced, and however, in most cases, this decrease was unreliable. Therefore, nociceptive afferentation from a damaged joint exerts protective inhibition on the muscles controlling the joint, which is expressed differently in different heads of complex muscles. To a greater extent, such inhibition affects the muscles directly involved in the joint, but can also affect the activity of other muscles. TSM is not involved in the movements of the hip joint.

Conclusions: Therefore, a decrease in EA in patients in is expressed to a much lesser extent. That is, the farther the examined muscle is from the affected area, the less effects of nociceptive irritation affect it. However, a significant decrease

in the amplitude of arbitrarily induced EA was nevertheless observed in the soleus head of the TSM. Nociceptive impulses from the affected joint entering the spinal cord spread up and down the intraspinal paths, inhibiting the motor centers of many, and especially the slow tonic muscles.

54ASM-0211 | Iron use in internal medicine: when and how. Is it worth?

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Iron deficiency anaemia (IDA) represents one of the five leading causes of years lived with disability in humans, and the top cause in women. Emerging evidence on the role of IDA in worsening clinical outcomes continues to accumulate in internal medicine settings, which prompted careful consideration of IDA management in international practice guidelines. In patients with chronic inflammatory conditions, the impact of IDA can be severe leading to disease exacerbation and deterioration. This is particularly relevant in elderly patients with multiple comorbidity, when even mild anaemia can be associated with increased mortality. IDA is a negative prognostic factor in chronic heart failure associated with disease progression and increased cardiovascular mortality; in chronic kidney disease, anaemia is commonly associated with reduced quality of life and there is a cumulative increase in the risk of predialysis mortality or development of end stage renal disease. IDA is finally the most common extra-intestinal manifestation in inflammatory bowel diseases. Although effective means for iron supplementation exist, making the right choice between oral and intravenous iron formulations is important to avoid unnecessary delays in iron repletion and correction of anemia. Oral iron is often poorly tolerated, with up to 70% or more of patients noting gastrointestinal issues; this may affect adherence to therapy. In addition, many patients will not respond to oral iron due to their underlying illness. Intravenous iron is being used more frequently to replete iron stores. True anaphylaxis is very rare, but complement-mediated infusion reactions may be seen in up to 1 in every 200 patients. Actually, iron repletion of deficient patients is one of the most gratifying treatments that can prescribed in internal medicine; the use of oral and intravenous iron has increased dramatically with benefits in order to avoid the adverse effects of iron deficiency.

54ASM-0237 | Iron-deficiency anemia & H. Pylori infection: current guidelines

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Iron deficiency is a major health problem worldwide, and affects especially women of childbearing age, children, and individuals living in low- and middle-income countries. The prevalence of iron-deficiency anemia (IDA) in USA is higher in females and ranges 1-5% overall. There are several causes of IDA, including reduced iron absorption from the upper gastrointestinal tract. The most clinically important disorder affects the mucosal cells, responsible for iron absorption and include, among the others, celiac disease, atrophic gastritis, and bariatric surgery.

H. pylori is a human pathogen transmitted from human to human. The germ causes chronic active gastritis in all colonised subjects. Possible evolutions of *H. pylori* infection include peptic ulcer disease, atrophic gastritis, gastric adenocarcinoma, and MALT (mucosa-associated lymphoid tissue) lymphoma. *H. pylori* eradication cures gastritis and can alter the progression to long-term complications, or recurrence of disease. Thus, *H. pylori* is an infectious disease irrespective of an individual's symptoms and stage of disease. Several evidences in adults and pediatric populations link *H. pylori* infection to unexplained IDA, idiopathic thrombocytopenic purpura (ITP), and vitamin B12 deficiency. In patients with IDA, the prevalence of *H. pylori positivity* ranges from 14% to 18%. Notably, patients with iron-deficiency anemia due to *H. pylori* infection may show a refractory response to oral iron treatment, while H. pylori eradication improves anaemia and increases haemoglobin levels. The effect is more evident in those with moderate to severe anaemia. Thus, recent guidelines, suggest that patients (even pediatric) with recurrent IDA, a normal esophagogastroduodenoscopy and colonoscopy, should undergo *H. pylori* testing and eradication (1-3). Local surveys, however, point to inappropriate, untargeted and vast use of urea breath test for diagnosing *H. pylori* infection, as well as to unappropriate use of therapeutic regimens for eradication, with waist of economical, and health resources (4). Given the burden of IDA, the potential link of IDA to *H. pylori* infection and gastritis, the possibility to diagnose the infection quickly, either noninvasively or endoscopically, and the therapeutic regimens available for eradication, different professional profiles (family doctors, patients, specialists, and referral centres) should better implement effective diagnostic and therapeutic strategies.

References: 1. P. Malfertheiner, Gut 66 (1), 6-30 (2017).
2. W.D. Chey, Am J Gastroenterol 112 (2), 212-39 (2017).
3. N.L. Jones, J Pediatr Gastroenterol Nutr 64 (6), 991-1003 (2017).

4. A. Di Ciaula, J Gastrointestin Liver Dis 26 (2), 129-37 (2017).

54ASM-0322 | Cytochrome P-450 in tumors and mammary adipose tissue in patients with breast cancer

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Background: The risk of developing of the breast cancer can be determined by genetic, metabolic factors and lifestyle characteristics, which can alter the metabolism of sex hormones. Cytochrome P-450 (CYP) is an oxygenase containing a heme-iron center that is involved in the biosynthesis and metabolism of estrogens, is a tumor response to the antiestrogen therapy. Determining the role of Cytochrome P-450 in the etiology of this disease can provide critical information for patient.
Materials and Methods: The studies were performed on 65 tissue samples of tumors, 49 mammary adipose tissue (MAT) taken at 5 cm from the tumor node and 49 samples of the MF of tissue in contact with the tumor of breast cancer patients with a BMI of less than 25 kg/m^2 and more 25 kg/m^2. The levels of the oxidized and low-spin cytochrome P-450 and its isoform CYP19A1 were studied by electron paramagnetic resonance (EPR) and spectrophotometric methods. Immunoblotting was performed in accordance with standard protocols with antibodies against CYP1A2 and CYP19A1, GAPDH in accordance with Helsinki declaration.
Results: Accumulation of MAT in tumor tissues changes the activity of cytochrome P-450 CYP19A1, especially its low-spin form, and leads to the increase of the levels of oxidized ($g = 2.42$) and low-spin ($g = 2.25$) forms of cytochrome in tumor cells. It correlates with BMI $\leq$ 25 kg/m^2 and $\geq$ 25 kg / m^2. It was also found that during the formation of the P-450 complex of CYP19A1 with androstenedione in MAT of patients with breast cancer, intense EPR signals with g-factors of 8.1 and 3.28 appear which can be a marker of the metabolic activity of the enzyme. An increase in the levels of CYP1A2 in tumor cells correlates with elevated levels of 'free' radicals in the mitochondria, the type of cells and the stage of the disease, and indicates that CYP1A2 can affect the energy metabolism of breast cancer cells and enhance cellular hypoxia.
Conclusions: Detection of the high levels of cytochrome P-450 activity in tumors and adipose tissues of patients with breast cancer may have potential use as a biomarker in deciding the use of personalized treatment regimens. Detection of increased activity of CYP1A2 and CYP19A1 in breast cancer may be a risk factor for the progression of breast cancer.

S14 – TRANSITIONAL, TRANSLATIONAL ASPECTS AND GENETICS OF FAMILIAL MEDITERRANEAN FEVER

54ASM-0223 | Onomastic heritage of Jewish origin in Altamura (Bari). The current state of research

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In Puglia and Basilicata, as indeed throughout the Kingdom of Naples, Israelites of Jewish religion and converted (the so-called neofiti o cristiani novelli) constantly coexisted. There are numerous documents that tell and describe the life of Jews and new Christians in the analyzed territory. However, it is difficult to pinpoint the path of a converted Jew. When baptized, Hebrew names and surnames were transformed, translated or readapted into 'Latin' names. Imagine an individual who changes identity overnight. The study I am conducting starts fifteen years ago. It is focused on demonstrating that much of the current cognominal heritage (and therefore the families bearing those surnames) of Altamura, a populous and important city in the province of Bari, has Jewish origins. My theory comes from numerous clues that gravitate particularly in the field of onomastics. In 1523 and then in 1527 the city of Altamura was hit by a terrible wave of plague. Chroniclers of that time reported that a large part of the population died in that circumstance and that the city was later repopulated by people from neighboring territories. The name-day heritage of these new inhabitants, collected through the study and unedited analysis of parish registers, notarial deeds, Stati delle Anime (censuses), Catasti Onciari, reveals a clear Jewish origin. The names (and in many cases the surnames) brought by the inhabitants of the city of Altamura, in fact, do not seem to derive from the Christian tradition, but Jewish (names brought by the new Christians present in the consulted documents or in the sacred books of the Jewish religion). A datum, the latter which, together with the others, is attempting to analyze, study, interpret. At present the research conducted by me requires a comparison with qualified experts in Jewish history and onomastics, so

that we can come up with a thesis that has as much a scientific guise as possible.

54ASM-0232 | The assessment of pathogenicity of mefv gene variants and its translation into clinical practice

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Familial Mediterranean Fever (FMF) is the most common among the hereditary recurrent fevers (HRF) with an estimated prevalence of 1 in 5000. FMF it is caused by mutations in the *MEFV* gene encoding for the pyrin protein. While originally described as an autosomal recessive disease, recent reports revealed that, at least in some families, the trait appears to be transmitted as autosomal dominant. Further, the presence of FMF symptoms in individuals carrying a single *MEFV* mutation is still debated. Therefore, in FMF diagnosis, it is important to precisely and reliably assess the impact of variants identified in patients. We recently reported FMF families presenting either autosomal recessive inheritance or apparent autosomal dominant transmission (1). In one of these families we revealed a truncating frameshift mutation *in-cis* with a missense *MEFV* mutation. This rare genotype lend support to the hypothesis that FMF symptoms are due to variants with hypermorphic effects and possibly also gene-dosage mechanisms. Differently from the other three major genes responsible for different HRFs (*TNFRSF1A, NLRP3* and *MVK*), many *MEFV* missense variants listed at (https://infevers.umai-montpellier.fr/web/) are still classified as variant of unknown significance (VOUS). Thus, in many cases the genetic referral is inconclusive causing an FMF diagnosis which cannot be confirmed. To reduce the number of unclassified variants we applied machine learning methods to train prediction algorithms with missense variants definitively classified as benign or pathogenic. Using this strategy, we could successfully reclassify 96/133 missense VOUS variants as leaning benign (N = 61) or leaning pathogenic (N = 35). Further, we could separate missense variants in the three sharply divided categories LIKELY BENIGN, VOUS AND LIKELY PATHOGENIC. In addition, we confirmed that *MEFV* variants with a putative pathogenic role cluster in the PRY, SPRY domains of the pyrin protein, while LIKELY BENIGN and VOUS are spread throughout the coding sequence of the pyrin protein (2). Finally, the same methodology can be reliably applied to fine tune any prediction tools geneticists may want to implement in their variant interpretation pipeline for improved variant classification (3).

References: 1) Stella A. et al. Familial Mediterranean fever: breaking all the (genetic) rules. *Rheumatology*. (2019) 58:463-467
2) Accetturo M. et al. Improvement of MEFV gene variants classification to aid treatment decision making in familial Mediterranean fever. *Rheumatology*. (2019) Aug 14. https://doi.org/10.1093/rheumatology/kez332.
3) Accetturo M, Bartolomeo N, Stella A. In-silico Analysis of NF1 Missense Variants in ClinVar: Translating Variant Predictions into Variant Interpretation and Classification. *Int J Mol Sci*. 2020;21(3):E721.

54ASM-0259 | FMF and PFAPA syndrome in two Armenian siblings: A case report

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Background: Familial Mediterranean Fever (FMF) and syndrome of periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) are the most common auto inflammatory diseases. FMF is characterized by recurrent attacks of fever and polyserositis, usually lasting 1-3 days. It is caused by mutations in the MEFV gene. PFAPA is sporadic, and no ethnic disease. It is lasting 3-7 days with recurrence every 3-8 weeks. There are some clinical and therapeutic similarities of FMF and PFAPA syndrome.

Materials and Methods: To describe siblings suffering FMF and PFAPA, characterize their association according clinical, laboratory and therapeutic aspects.

The diagnosis of FMF established according criteria Tel-Hashomer and genetic investigation. PFAPA syndrome was diagnosed considering clinical criteria, steroid response and excluding infection causes of tonsillitis.

Results: 2-year-old boy diagnosed FMF according clinical, genetic (MEFV- V726A/V726A) and examination results during the attacks: leukocytosis, elevated CRP and ESR, pleural effusion, pericarditis. FMF was established. The treatment with colchicine was started (0.25/0.5 mg/day) gradually increased the dose during the follow-up. The episodes of high fever with exudative tonsillitis, cervical lymphadenopathy was started from 9 years old under the 1.0 mg/d colchicine treatment. Duration of attacks was 5-6 days without respond to nonsteroidal anti-inflammatory drugs. A single dose of betamethasone (0.15 mg/d) dramatically aborted fever and exudates in a few hours. FMF with PFAPA was established.

8-year-old boy admitted to the hospital two times, complains of: high fever, abdominal and thoracic pain, exudative tonsillitis, lymphadenopathy. Recurrence of attacks - every

4 weeks, duration of attacks - 5-6 days without respond to nonsteroidal anti-inflammatory agents. Examination revealed: plural effusion, cervical adenopathy - 18-19 mm, leukocytosis, elevated CRP and ESR. Genetic investigation of MEFV: V726A/N mutation. A single dose of betamethasone dramatically aborted exudates and fever. PFAPA syndrome with FMF was established and treatment with colchicine was started 1.0 mg/d. It should be noted, that we gradually increased the dose of colchicine up to 1.5 mg/d for two siblings (under the 1.0 mg/d attacks continued) which showed it dramatic results without any side effects.

Conclusions: Our follow-up presume, that a comorbidity of two diseases require more higher dose of colchicine. For the understanding peculiarities of coexistence of the above diseases and treatment strategies dose of colchicine, the prospective comparative studies on large cohorts of patients with and without endemic origin of FMF are needed.

54ASM-0263 | Concept of signal transduction in FMF family: A case report

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Background: Familial Mediterranean Fever (FMF) is characterized by recurrent attacks of fever, peritonitis, pleuritis, arthritis. Peritonitis is the most common clinical picture of FMF. The frequent association of FMF with other disorders is remarkable and of great interest.

Materials and Methods: To discover case of FMF complicated of ASBO and describe coexistence of FMF with endocrinological and gynecological disturbances in the same family. The diagnosis of FMF established according criteria Tel-Hashomer and genetic investigation. Coexistence other diseases with FMF was diagnosed according clinical and laboratory results. We did not find any members of FMF in family tree.

Results: 12-year-old boy admitted to the 'Arabkir' JMC surgical department with complains of: severe abdominal pain, vomiting, fever. Examination results: WBC - 17.12*10.^{9}, HGB – 76 g/l, HCT-22.4%, PLT-195*10.^{9}, CRP -20.30 mg/dl, PT- 70%, INR-1.32. ESR-8 mm/h. Small intestinal coils with the horizontal fluid level were discovered in abdominal X-ray. Dilated intestinal loops were detected in abdominal ultrasonography. The adhesive small bowel obstruction (ASBO) was established. MEFV revealed M694V/M680I mutations with high level of SAA protein - 167.1 μg/mL (N<8). FMF was established and treatment with colchicine was started by 1.5 mg/day.

14-year-old girl admitted to the FMF department with complains of: fever, abdominal and chest pains, with few hours menstruation from 12 years. Recurrence of attack was 1-2 time per month, with 1-2 day duration. Examination during the attack: leukositosis with elevated CRP, ESR, pleural reaction, fluid in the abdomen with enlargement abdominal lymphatic nodules (18 mm). The endocrinological and gynecological investigations reveled hypoplasia of uterus with low level genital hormones and low level vitamin D – 12.12 ng/mL(N>30). MEFV reveled M694V/M680I mutation, SAA protein 6.2 μg/mL. FMF was established, colchicine was started with 1.0 mg/d (during the follow-up increasing the dose). Hypoplasia of uterus and association with secondary hypogonadism we established according clinical and laboratory findings.

Conclusions: This is a very interesting case presenting relationship between FMF and ASBO, coexistence of FMF with hypoplasia of uterus, association with secondary hypogonadism and hypovitaminosis D in the same family, therefore, surgical department should be kept in mind as a possible complication of ASBO especially in family cases of FMF. However, we suppose, that these diseases are connected not only with the relationship of geneses, but also with major pathological development in mesenchymal tissue in morphogenesis. We do not exclude that the manifestation of the above mentioned diseases can be explained by the concept of Signal Transduction.

S15 – NUTRITION BIOLOGY AND PATHOBIOLOGY

54ASM-0070 | New milk-derived bioactive peptides: antioxidant effects through Keap1/Nrf2 pathway activation

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Background: During the last decades, chronic and age-related diseases, such as diabetes, cardiovascular diseases and obesity gained increasing relevance. To counteract the alteration of the inflammatory process and an imbalance of cellular redox status, natural compounds, as bioactive peptides, acquired interest. This study was focused on the analysis of the antioxidant properties of new milk-derived bioactive peptides in Caco-2 cells in order to understand their mechanism of action.

Materials and Methods: Antioxidant peptide enriched fractions were extracted from fermented milk and purified. Once

the sequence of twenty-three peptides was identified through mass spectrometry, they were synthesized and tested *in vitro* and in a cellular model for their antioxidant properties. Four peptides, N-15-M, E-11-F, Q-14-R and A-17-E, were selected for their protective effects against oxidative stress induced by TbOOH, both as rescue of the viability and inhibition of ROS production. All cellular assays were performed using Caco-2 cells treated for 24 hours with the peptides.

Results: The mechanism of action of the four selected antioxidant peptides in the cell environment was studied. N-15-M, Q-14-R and A-17-E were able to determine the activation of Keap1/Nrf2 system in Caco-2 cells, resulting in Nrf2 translocation from the cytosol to the nucleus, leading to the transcription of antioxidant enzymes. For this reason, the gene expression of TrxR1, GR, NQO1 and SOD1 was performed by RT-PCR and confirmed by WB analysis in cells treated with the four bioactive peptides. N-15-M, Q-14-R and A-17-E, the three peptides that activate Keap1/Nrf2 pathway, increased both the expression and the activity of the antioxidant enzymes in Caco-2 cells. Molecular docking approach of Keap1 with the peptides was perform, showing the interaction of N-15-M, Q-14-R and A-17-E with the residues of the Keap1 pocket involved in the binding with Nrf2.

Conclusions: The obtained results suggest that these new bioactive peptides act as antioxidants by disrupting Keap1/Nrf2 interaction.

54ASM-0080 | Bioscreening of bis(2-pyridyl)-3-(1,2,4-triazolyl)propane on the pain sensitivity of male and female rats

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Background: The aim of the work is to conduct preclinical bioscreening of bis(2-pyridyl)-3-(1,2,4-triazolyl)propane (BTP) on the pain sensitivity of male and female rats.

Materials and Methods: 60 male and 60 female Wistar rats weighing 180-200 g were used. We selected 6 groups of males and females (10 individuals in the group). The control group was administered intraperitoneal 0.9-% NaCl solution; the five experimental groups were given BTP in doses from 5 to 200 mg/kg, respectively. Bioscreening effects of BTP on the rats pain sensitivity was performed 1 hour after injection in the tests 'tail-flick' (ANALGESY-METER LE 7106, PanLab Harvard Apparatus) and Randall-Selitto (BIO-RPR, Bioseb). The significance of differences between the groups was determined by ANOVA with aposteriori test Tukey and Dunn's multiple comparison criterion.

Results: The 'tail-flick' test revealed analgesic effect of BTP at a dose of 200 mg/kg in males, and at doses of 5 and 50 mg in females, expressed in a significant increase in the latent period of tail retraction relative to control by 25.46, 40.7 and 41% ($P \leq 0.05$), respectively. In the Randall-Selitto test analgesic effect of BTP was detected in males (50 mg/kg) and females (5 and 50 mg/kg). It was manifested in a significant increase in the pain threshold in response to mechanical compression of the tail with 'forceps' relative to control at 26.9% (males 50 mg/kg, $P \leq 0.05$), 39.4 (females 5 mg/kg, $P \leq 0.05$) and 45.3% (females 50 mg/kg, $P \leq 0.001$), respectively. The most pronounced analgesic effect was manifested in a dose 50 mg/kg.

Conclusions: Thus, the analgesic effect of BTP is mainly manifested in doses 5 and 50 mg/kg, and the most pronounced effect was manifested at a dose 50 mg/kg. Females were more sensitive to the action of BTP. Since 1,2,4-triazoles are a convenient matrix for assembling biologically active coordination compounds, the synthesis of new analgesics based on BTP is promising.

54ASM-0150 | Association of VDR gene FokI polymorphism with circulating vitamin D level in breast benign disease patients

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Background: Vitamin D deficiency and breast benign disease (BBD) are considered to be an independent risk factors for the breast cancer. It's been shown that VDR gene polymorphism associated with deficiency of vitamin D (VD) in tissue but its effect on the level of circulating forms is still unclear. In recent work we study association between VDR gene polymorphism (rs2228570) and circulating forms of VD in the blood in healthy women and patients with benign breast diseases.

Materials and Methods: Genotyping was performed by real-time PCR using TaqMan assay (OOO SibDNA, Russia) on DNA samples from 216 unrelated women: 81 patients with diffuse mastopathy (DFM), 35 patients with fibroadenoma (FA) and 100 women of control group. The diagnosis of BBD was proved by mammary glands ultrasound examination and digital mammography, confirmed by puncture biopsy and histological examination of tissues after sectoral

resection of the mammary gland. VD level was determined by ELISA (Abbott, USA). Statistical analysis done with packet program R.

Results: Alleles and genotypes in all studied groups were according to HWE ($P > 0.05$). Further analysis showed that VDR polymorphism did not have any significant influence on circulating VD level in patients with FA and healthy women ($P > 0.05$). However, DFM patients, carriers of allele T and genotype TT were characterized by significant low VD level compared to the CC genotype carriers (median 17.2 ng/mL and 20.1 ng/mL, respectively, $P = 0.04$). Patients with heterozygote genotype were characterized by a tendency to decreasing of circulating VD level compared with CC homozygotes (median 17.6 ng/mL, $P = 0.069$).

Conclusions: Effect of VDR FokI polymorphism on the level of the circulating form of vitamin D may be one of the pathogenesis links that increases the individual risk of developing breast cancer especially in women with DFM but this statement needs further confirmation.

54ASM-0151 | Influence of vitamin D deficiency correction on the density of the mammary glands in women with a diffuse form of mastopathy

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Background: High radiological density of the mammary glands and vitamin D (VD) deficiency are common risk factors for the breast cancer and diffuse form of mastopathy (DFM). Treatment of DFM is in priority for the primary prevention of breast cancer. That's why we estimated effectiveness of vitamin D deficiency correction in patients with DFM according to the dynamics of X-ray mammography.

Materials and Methods: 92 women with DFM and the 124 healthy women were underwent X-ray mammography with density assessment according to the classification of the American College of Radiology (ACR). All patients started vitamin D treatment with a 5000 IU decreasing maintenance dose of 2000 IU during 6 months. The level of the circulating form of VD serum was measured by the CMIA.

Results: The majority of women with DFM had a VD deficiency compared to a healthy women (97.8% and 7.3%, respectively). High density of the mammary gland (ACR3 + ACR4) was diagnosed in 52% patient with DFM while the healthy women had a low density (ACR1 + ACR2, 93%) values. VD provision and ACR showed the lower VD levels in women with high density compared to patients with low density. The dynamics of the breast X-ray density during VD treatment after 6 months of therapy showed positive dynamics in decreasing of mammographic density in 67% women, lack of dynamics observed in 33% observations. Negative dynamics in the form of increasing breast density was not recorded in any of the cases of therapy.

Conclusions: Correction of vitamin D deficiency supposed to be an effective method for reducing the radiological density of the breast and the target level for the correction has to be not lower than 43 ng/mL of circulating form of vitamin.

54ASM-0176 | Polyphenolic extracts from Ephedra foeminea fruits exert protective effects against oxidative injury in human endothelial cells

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Background: Endothelium dysfunction is associated with many chronic diseases such as metabolic syndrome and atherosclerosis. Plant-derived polyphenols are natural antioxidants playing many protective effects against oxidative injury and related diseases. Here, we investigated the beneficial effects of different polyphenol-rich extracts of *Ephedra foeminea* fruits, a medicinal plant from the eastern Mediterranean area.

Materials and Methods: Dried *E. foeminea* fruits were extracted,, with ethanol (EE), methanol/water mixture (EMW), hexane (Ehex) or ethyl acetate/water (Epoly), alternatively. The phenolome profile of each extract was characterized using High Performance Liquid Chromatography-Mass Spectrometry (HPLC/MS) analysis. Total phenolic and flavonoid content, the scavenging properties and the toxicity of each extract were assessed spectrophotometrically. Then, using the human endothelial cells HECV we assessed the antioxidant and cytoprotective *in vitro* potential of Epoly by measuring apoptosis, ROS production, lipid peroxidation, mitochondrial membrane potential and nitric oxide (NO) release by spectrophotometric and fluorimetric assays.

Results: Epoly showed the highest phenol/flavonoid content and radical scavenging capacity. On H_2O_2-insulted HECV cells Epoly (25 µg/mL for 24 h) was able: *(i)* to counteract

the ROS production (-53%; $P \leq 0.01$), and lipid peroxidation (-39%; $P \leq 0.01$); *(ii)* to rescue the ROS-dependent decrease in the mitochondrial membrane potential (+46%; $P \leq 0.05$) and apoptosis induction; *(iii)* to restore endothelial cell viability and migration.

Conclusions: The findings indicated that the polyphenol-enriched extract (Epoly) from *E. foeminea* plays *in vitro* anti-oxidant and cytoprotective effects and might be used as nutraceutical for treating radical-related endothelium dysfunction and inflammation.

54ASM-0179 | Aquaporin-9 (AQP9) is involved in the lipid-lowering activity of the nutraceutical silybin on hepatocytes through modulation of autophagy

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Background: Hepatic steatosis is the hallmark of non-alcoholic fatty liver disease (NAFLD) and mainly originates from increased uptake of circulating long-chain fatty acids (FAs) in the hepatocyte. In the liver, excess FAs are esterified with glycerol to triglycerides (TGs) and stored as lipid droplets (LDs). So far, therapeutic options in NAFLD are lacking. The nutraceutical silybin has shown antioxidant, anti-inflammatory and cytoprotective actions and it has been preliminarily tested in patients with NAFLD.

Materials and Methods: Here, we studied the molecular mechanisms through which silybin may improve hepatic lipid dyshomeostasis using an *in vitro* model of NAFLD progression induced by sequential exposure of FaO hepatoma cells to FAs (simple steatosis, SS) and TNFα. (steatohepatitis, SH). For each condition, we assessed the FA profile of lipid droplets, the mitochondrial oxidation activity, the autophagy level and the glycerol import mediated by Aquaporin-9 (AQP9) by real-time PCR and/or immunofluorescence.

Results: In both models the lipid lowering activity of silybin was found to be associated to: (*i*) upregulation of AQP9 that mediates an increased uptake of glycerol by hepatocytes; *(ii)* reduction in the fat-stimulated autophagy through reduction of LC3II and Atg7 levels; *(iii)* stimulation of mitochondrial FA oxidation through upregulation of very long-chain acyl-CoA dehydrogenase (VLCAD) and uncoupling protein 2 (UCP2) expression, and stimulation of Cytochrome C oxidase (COX) activity. Interestingly, silybin modified the profile of FA stored in LDs by upregulating the stearoyl CoA desaturase (SCD1) mRNA expression resulting in increased levels of short/medium chain FA and decreased saturated/monounsaturated FA ratio.

Conclusions: The hepatoprotective effects of silybin intersect TG metabolism and AQP9-mediated import of glycerol through fat-induced autophagy in hepatocytes.

NUTRITION BIOLOGY AND PATHOBIOLOGY

54ASM-0189 | Effects of two chickpea accessions on hepatic lipid over-accumulation and NF-kB activity

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Background: Dietary habits play a role to prevent the development of lifestyle-associated diseases. Diets supplemented with chickpeas have numerous beneficial effects among which that of ameliorating the body lipid profile. The present study was carried out to evaluate the possible bioactive actions of two genetically and phenotypically different chickpea accessions selected from the germplasm collections as they belong to distinct genetic clusters: MG_13 and PI358934.

Materials and Methods: Rat hepatoma FaO cells treated with a mixture of free fatty acids-FFAs (0.5 mM oleate and 0.25 mM palmitate for 3 h) were used to mimic hepatic steatosis. In addition, an animal NAFLD model was made by feeding mice a high-fat diet (HFD). Briefly, mice were fed for 16 weeks with four kinds of diets: a control diet (CTR); a high-fat diet (HFD) which contained 45% fat; high-fat plus 10% of raw crushed chickpea seed flours replaced crude fibers and ashes.

Results: Both chickpea accessions displayed significant antioxidant ability in vitro as well as in vivo conditions.

However, only the accession MG_13 led to a significant reduction in the lipid accumulation in both steatotic FaO cells and liver parenchyma of HFD mice. Moreover, the hyperglycemia and AST levels of mice fed HFD supplemented of MG_13 resulted significantly lower than those measured in the HFD animals that did not receive MG_13. Interestingly, exposure to MG_13 prevented the phosphorylation of NF-KB, an inflammatory nuclear factor upregulated during HFD and linked to obesity.

Conclusions: Overall, between thet wo analyzed chickpea accessions only MG_13 showed beneficial effects on the cellular and animal NAFLD models used for the study. This finding underlines the importance of characterizing the healthy actions of chickpea accessions of global food interest.

54ASM-0390 | Effect of etidronic acid adduct bis (2-pyridyl)-3-(1,2,4-triazolyl)butane on male rats behavior in the 'open field' test

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Background: The aim of the work is to evaluate the effect of etidronic acid adduct and bis(2-pyridyl)-3-(1,2,4-triazolyl) butane (EA+BTB) on male rat behavior in the test 'open field' with a single administration.

Materials and Methods: One week before the experiment, 30 male Wistar rats (weight 180-200 g, FSUE Nursery of laboratory animals 'Rappolovo', Saint-petersburg, Russia) with average motor activity in the 'open field' test was selected. The experiments were performed on 3 groups male rats (10 individuals in the group). The control group was administered intraperitoneal 0.9-% NaCl solution; the two experimental groups were given EA+BTB in doses 5 and 50 mg/kg, respectively. Bioscreening effects of EA+BTB on the rats behavior was performed 1 hour after injection in the test 'open field' (IR Actimeter, PanLab Harvard Apparatus, Spain). Actitrack 2.0 software (PanLab Harvard Apparatus, Spain) used to manage the workstation and collect data. The significance of differences between the groups was determined by ANOVA with aposteriori test Tukey and Dunn's multiple comparison criterion.

Results: EA+BTB at a dose of 50 mg/kg significantly reduced the total distance traveled in male rats by 83.4% ($P \leq 0.001$, n = 10), and in the periphery of the field – by 64.5% ($P \leq 0.001$, n = 10). In this dose, EA+BTB significantly suppressed vertical motor activity by 90% compared to the control ($P \leq 0.05$, n = 10), including completely disappearing vertical rearings without support ($P \leq 0.001$, n = 10), and vertical rearings with support decreased by 83.4% ($P \leq 0.001$, n = 10). The results indicate a sedative effect of EA+BTB at a dose of 50 mg/kg in relation to the locomotor activity of animals. At doses of 5 and 50 mg/kg, the tested substance reduced overall research activity (RA) relative to control by 57 ($P \leq 0.05$, n = 10) and 63.3% ($P \leq 0.01$, n = 10), respectively, and peripheral RA at a dose of 50 mg/kg – by 56% ($P \leq 0.01$, n = 10). Data on the study of the exploratory behavior of male rats indicate the anxiogenic effect of EA+BTB on the behavior of male rats at doses of 5 and 50 mg/kg in the open field test.

Conclusions: EA+BTB has a sedative effect at a dose of 50 mg/kg and an anxiogenic effect when administered once at doses of 5 and 50 mg/kg on the behavior of male rats in the open field test. This substance is promising for further preclinical tests as a brake agent of the nervous system during course administration.

The reported study was funded by Russian Science Foundation according to the research project № 18-13-00024.

54ASM-0395 | Effects of 1-hydroxyethane-1,1-diphosphonic acid adduct and bis(2-pyridyl)-3-(1,2,4-triazolyl)butane on thermal pain sensitivity of male and female rats

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Background: The aim of the work is to evaluate the effect of 1-hydroxyethane-1,1-diphosphonic acid adduct and bis(2-pyridyl)-3-(1,2,4-triazolyl)butane (HDA+BTB) on thermal pain sensitivity of male and female rats in various doses.

Materials and Methods: Experiments were performed on 30 male and 30 female Wistar rats weighing 200-220 g. Two experimental groups of males and two experimental groups of females (n = 10 in each group) received single intraperitoneal injections of 0.2 mL HDA+BTB solution in doses of 5 and 50 mg/kg, respectively. The males (n = 10) and females (n = 10) of control groups of animals received single intraperitoneal injections of 0.2 mL of 0.9% NaCl solution with a volume of 0.2 mL. Thermal pain sensitivity of animals was assessed 1 hour after a single intraperitoneal injection of the studied solutions by 'tail-flick' (LE7106 Tail-flick Meter, Pan Lab Harvard Apparatus, Spain) and 'hot plate' (Cold and hot plate CHP, Bioseb, France) tests. The significance of differences between the groups was determined by ANOVA with aposteriori test Tukey and Dunn's multiple comparison criterion.

Results: In the 'tail-flick' test in male rats, HDA+BTB in a dose of 50 mg/kg caused a significant increase in the latent

period of tail retraction by 19.5% ($P \leq 0.05$, n = 10) relatively to control (n = 10). This indicates the presence of an analgesic effect of HDA+BTB in a dose 50 mg/kg, implemented with the participation of the perceptual component of nociception and the spinal mechanism for regulating pain sensitivity. In female rats this indicator has not changed significantly.

In the 'hot plate' test in male rats, HDA+BTB in a dose of 50 mg/kg significantly increased latent period of pain reaction by 57.1% ($P \leq 0.01$, n = 10) compared to the control (n = 10). In female rats HDA+BTB in a dose of 5 mg/kg also significantly increased latent period of pain reaction by 37.8% ($P \leq 0.01$, n = 10) relatively to control (n = 10). This indicates the participation of supraspinal mechanisms in the thermal pain sensitivity.

Conclusions: It was found that HDA+BTB in doses significantly changes the thermal pain sensitivity of male and female rats in thermal pain tests ('tail-flick' and 'hot plate'), showing gender specificity of the analgesic effect with the participation of various pain mechanisms and nociception components. This compound recommended for further preclinical tests of its analgesic activity.

A possible reason for the gender specificity of HDA+BTB analgesic effect is the different tolerance of receptors and structures of the central and peripheral nervous system in males and females rats to pain, and participation of various nociception components.

S16 – TRANSLATIONAL ALLERGOLOGY

54ASM-0403 | Immunology and mammary cancer development: addressing the role of mast cells

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Background: Mammary cancer is one of the most frequent cancers worldwide. Mast cells are among the cells of tumor microenvironment and have been associated with increased angiogenesis and poor prognosis. Despite this, the role of mast cells on mammary cancer is not fully elucidated. In this way, this work studied the role of mast cells in a rat model of mammary cancer chemically-induced.

Materials and Methods: All experiments were performed in accordance with the Portuguese and European legislation on the protection of animals used for scientific purposes. The experiments were approved by the Portuguese (no.008961) and University (CE_12-2013) Ethics Committees. Thirty-four female Sprague-Dawley rats were randomly divided into five experimental groups. At seven weeks of age, mammary tumors' 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). Groups II and IV (n = 2) were treated with ketotifen in drinking water (1 mg/kg/day, 7 days/week) immediately after the MNU administration for 18 weeks, while the group III received the ketotifen after the development of the first mammary tumor. 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 formalin for posterior analysis. Tumors' vascularization, proliferation and apoptosis were also assessed by immunohistochemistry (Vascular Endothelial Growth Factor (VEGF)-A, Ki-67, and caspase-3 and caspase-9).

Results: Animals from groups IV and V did not develop any mammary tumor. Twenty-one animals (six animals from group I, eight animals from group II and seven animals from group III) developed a total of 58 mammary tumors, mainly classified as papillary non-invasive carcinomas. Tumors' vascularization was similar among groups ($P > 0.05$). Mammary tumors from group II exhibited the lowest proliferation ($P < 0.05$) and apoptotic indexes.

Conclusions: The mainly positive effect of the ketotifen administration seems to be the reduction of tumor proliferation when the drug was administered before mammary tumor development.

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S17 – PATHOPHYSIOLOGY AND TREATMENT OF COVID-19

54ASM-0391 | Bioscreening effects of 1-hydroxyethane-1,1-diphosphonic acid adduct and bis(2-pyridyl)-3-(1,2,4-triazolyl)butane on female rats behavior in the 'open field' test

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Background: The aim of the work is to evaluate the effect of 1-hydroxyethane-1,1-diphosphonic acid adduct and bis(2-pyridyl)-3-(1,2,4-triazolyl)butane (HDA+BTB) on female rat behavior in the test 'open field' with a single administration.
Materials and Methods: The experiments were performed on 3 groups female Wistar rats (10 individuals in the group) with average motor activity and weight 180-200 g in the 'open field' test. The control group was administered intraperitoneal 0.9-% NaCl solution; the two experimental groups were given HDA+BTB in doses 5 and 50 mg/kg. Bioscreening effects of EA+BTB on the rats behavior was performed 1 hour after injections in the test 'open field' (IR Actimeter, PanLab Harvard Apparatus, Spain). Actitrack 2.0 software (PanLab Harvard Apparatus, Spain) used to manage the workstation and collect data. The significance of differences between the groups was determined by ANOVA with aposteriori test Tukey and Dunn's multiple comparison criterion.
Results: In female rats, HDA+BTB showed a sedative effect at a dose of 5 mg/kg, significantly reducing the total distance traveled by 62.6% ($P \leq 0.05$, n = 10) and the distance traveled at the periphery by 51.3% ($P \leq 0.05$, n = 10) compared to the control. In the same dose, HDA+BTB reduced the number of vertical rearings without support in female rats to zero, and racks with support – by 73.3% ($P \leq 0.001$, n = 10). There was no significant effect on the indicators of research behavior in any of the doses of HDA+BTB. The observed changes indicate a sedative and anxiogenic effect of HDA+BTB in female rats at a dose of 5 mg/kg.
Conclusions: HDA+BTB has sedative and anxiogenic effects at a dose of 5 mg/kg at single injection on the behavior of female rats in the 'open field' test. This substance is promising for further preclinical tests as a brake agent of the nervous system during course administration.
The reported study was funded by Russian Science Foundation according to the research project № 18-13-00024.

54ASM-0397 | COVID-19: the crucial role of internal medicine and geriatrics in the management of a complex disease

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Background: Italy has been particularly affected by the COVID-19 pandemic, with 239,706 infected subjects and 34,678 deaths on June 25, 2020. The multi-organ implications of the SARS-CoV-2 infection and the worst outcomes in fragile patients can valorize the role of internists.
Materials and Methods: We explored the role of a COVID-19 'grey-area' managed by specialists in internal medicine and activated in a large hospital in southern Italy (Policlinico di Bari, Apulia region), which served a metropolitan area of 1.3 million inhabitants.
Results: Before the outbreak, the Clinica Medica 'A. Murri' was a typical academic division of Internal Medicine. Until March 16, a total of 383 new infected subjects and nineteen COVID-19-related deaths were recorded in the Apulia region. On the same day, all COVID-19 negative in-patients were discharged from our unit or transferred to other medical wards. The staffs of two internal medicine divisions (one academic and one non-academic) were merged and re-located in a 'grey zone' equipped with 64 beds, in a building entirely dedicated to COVID-19. As internists, the specific mandate was the screening of a wide range of patients received from the ER, suspected for SARS-CoV-2 infection. Confirmed negative patients were transferred to specific COVID-19 negative wards or discharged. Positive patients were clinically managed in the unit, transferred (depending on the clinical evolution) to other COVID-19 wards, to post-acute facilities in the territory or placed in quarantine at home, after receiving full instruction on preventive measures. On June 26, a total of 770 patients were screened in this grey-area and 151 COVID-19 positive patients were clinically managed, with the worst outcome (death) in a minority of cases (13/151 subjects, 8.6%).
Conclusions: In conclusion, this organization facilitated the initial management of both non-COVID-19 and COVID-19-patients, and decreased the burden of patients entering other COVID-19 dedicated wards (i.e. ICU, infectious diseases). Due to the most severe clinical presentations and the highest risk of death in the elderly and/or in fragile patients, internists can play a critical role, since typically trained to manage the complexity of systemic diseases, and to coordinate a multidisciplinary approach.