In Argentina, Hemolytic Uremic Syndrome (HUS) caused by Shiga toxin (Stx)-producing *Escherichia coli* (STEC-HUS) infection is an endemic disease and one of the most common causes of acute kidney injury in children. Negligible amounts of free toxin are present in the circulation during HUS, and it circulates mainly bound to blood cells and microvesicles (MVs). Platelets and leukocytes derived MVs were detected in the plasma of STEC-HUS patients. In this sense, preliminary, we successfully identified circulating MVs carrying Stx2 (MVs-Stx2) in two STEC-HUS patients. In this study, our objective was to analyze the presence of MVs-Stx2 in blood samples of six STEC-HUS patients, between 3 and 17 years old, that were admitted to the Hospital Penna-Bahía Blanca. Blood samples were collected and underwent sequential ultracentrifugation to obtain a suspension enriched with MVs. Then, MVs were labeled with Annexin V-FITC and MVs-Stx2 were detected by a monoclonal anti-Stx2 antibody and a secondary antibody conjugated to Alexa Fluor 647. Finally, MVs were analyzed by flow cytometry. Data are expressed as the percentage of positives MVs-Stx2. Ten age-matched healthy controls were also recruited and a cut-off point for MVs-Stx2 was established. Medical history of patients indicated that to the day of hospitalization, three of them exhibited aqueous diarrhea, two showed bloody mucous diarrhea and the other had no diarrhea. For Stx2 PCR analysis, 50% of them were positive. Anti-LPS antibodies were positive for O145 IgM/IgG in three patients and for O157 IgG in one of them. All patients showed a significant percentage of MVs-Stx2 in circulation compared to controls (3.4%, 7%, 13.7%, 6%, 3.6% and 13.8% vs 1.76 ± 0.74 %, n=10, ($p<0.05$). Thus, these patients had MVs-Stx2 in their blood circulation on admission to the hospital. Detection of circulating MVs-Stx2 in patients infected with STEC could be useful for the early identification of patients at a high risk of developing HUS.

176. 462. **SIMULTANEOUS DETECTION OF THREE RESPIRATORY VIRUSES BY A SINGLE QPCR AMPLIFICATION FOLLOWED BY HIGH RESOLUTION MELTING ANALYSIS**

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After pandemic confinement, other respiratory viruses, such as Influenza and RSV, have started to circulate again. Since acute symptoms, overlap with those of SARS-CoV-2, molecular methods are required to identify the infecting virus. Accordingly, many research groups worked on the development of specific, rapid and cost/efficient methods to detect these viral infections. Most methods were developed on probe based qPCR techniques, strategies that requires expensive reagents. The aim of this work was to develop a diagnostic method based on a multiplex qPCR-HRM to identify specific sequences of RSV, H1N1 and SARS-CoV-2. M&M: synthetic targets of DNA sequences were designed to detect SARS-CoV-2 (103bp); Influenza (H1N1) (98 bp) and RSV (111bp). Online tools were employed for primer design and melting temperature (Tm) simulations. Quantitative PCR was carried out in multiplex reaction, mixing RSV, H1N1 and SARS-CoV-2 primers and SYTO9™ intercalating dye to visualize the progress of the qPCR/HRM melting. Results: Based on the GC content and amplicons length it was possible to detect the tested viruses. Specific viral sequences displayed differential Tm: RSV 76.1°C, H1N1 80.7°C and SARS-CoV-2 83.8°C. The sensitivity was assessed with serial dilutions of synthetic targets. Detection limit was up to 10 copies/ul for SARS-CoV-2 and H1N1, and 100 copies/ul for RSV. Simulated co-infections were efficiently detected. A preliminary assay with cDNA from nasopharyngeal swabs of patients previously diagnosed were concordant. Conclusion: This technique was able to discriminate three respiratory viruses, with high sensitivity and reproducibility. Preliminary tests involving positive samples showed result consistent with our approach. The next steps will include testing an extended number of diagnosed patients and optimize the reaction by coupling with a previous RT-PCR step. This development constitutes a simple and cost-effective strategy for viral diagnosis.

177. 643. **METFORMIN ANTHELMINTHIC EFFECTS ASSOCIATED WITH A DECREASE IN PARASITIC GLUCOSE AND GLYCOGEN IN VIVO**

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(*) equal contributions.

Most patients with cystic echinococcosis (CE) are diagnosed at advanced disease stages when the therapy with benzimidazoles is limited, being imperative new therapeutic approaches. Since in cestodes, glycogen is the main energy storage molecule and glucose the major fermentative substrate, we aim to target the energy production pathways in the parasite. Here, we assess the efficacy of metformin as an indirect AMPK agonist in experimental models of advanced CE, at 6 and 12 months post-infection, employing the highest dose of the drug tested (250 mg kg^{-1} day^{-1}) orally or intraperitoneally, respectively. Based on *in situ* detection of fluorescent glycogen with 2-NBDG, biochemical determinations of hydatid fluid, and biodistribution analysis of IRDye-800CW2-DG in mouse whole-body (using near-infrared fluorescent *in vivo* imaging combined with 3D optical tomography), we demonstrated that metformin increased glucose utilization in metacestodes through the glycolytic pathway, leading to the elevation of intracystic lactate levels (up to 7-10 mM), with a tendency to increase the glucose uptake in peritoneal cavity tissues of infected mice. Given that *Echinococcus* cells cannot oxidize lactate, it would diffuse to the surrounding tissue, activating signal transduction pathways involved in the differentiation of macrophages, fibroblasts and lymphocytes, and promoting the development of the cystic adventitial layer. By biochemistry and molecular approaches, we demonstrated intracystic accumulation of metformin at supra-pharmacological concentrations (>1 mM), which induced mitochondrial complex I inhibition and increased cellular Pi/ATP ratio, with AMPK activation and TOR inhibition (by dephosphorylation in Ser^{3122}) and, consequently, reduction of the Warburg effect and proliferation in germinal cells. In the context of normoglycemic mice, our results are in accordance with previous data showing glucose reduction in gut-liver crosstalk induced by metformin.

P1-METABOLISM & NUTRITION

WEDNESDAY 15TH NOVEMBER 9:00 - 10:30

CHAIRS: ROMINA HERMANN
ESTEBAN REPETTO

178. 61. **CHANGES IN BONE RESORPTION AND FORMATION MARKERS BY AGE AND DIFFERENT CONDITION THAT AFFECT BONE HEALTH. EXPERIMENTAL STUDY**

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Bone is constantly being remodeled by bone cells coordinated action. A delicate equilibrium between bone formation by osteoblasts and resorption by osteoclasts is needed to maintain mineral homeostasis and biomechanical integrity. Activity of these bone cells called bone remodeling is biochemically assessed by specific bone turnover markers of bone formation (osteocalcin or OCN) and bone resorption (CTX). Bone remodeling changes with age and is regulated by hormonal and nutritional factors. Objective: Evaluate bone remodeling in different life stages and conditions that impact bone metabolism: age, estrogenic status, dietary calcium (Ca) and/or vitamin D nutritional status (VD) in female Wistar rats. Animals were divided (n=8 per group) based on their age and estrogen deficiency: newborn (NB); weaning (RD); puberty (R45); young adult (R90 SHAM), late adulthood (R105SHAM), and estrogen deficiency (R90OVX, R105OVX) that fed a commercial diet. Effect of VD and/or Ca insufficiency was evaluated by feeding semi-synthetic diets formulated according to AIN'93-M modified in vitamin D (D): 100IU% or 0UI% (+D and -D, respectively) and/or calcium: 0.5% or 0.3% (0.5 and 0.3): R105OVX+D0.5; R105OVX-D0.5; R105OVX+D0.3, and R105OVX-D0.3. CTX and OCN were assessed by ELISA. ANOVA was used., p<0.05 was considered significant. Results (mean±SD) in the following order CTX (pg/mL) and OCN (ng/mL): RN: 83.9±3.5, and 2.49±0.19; RD: 70.5±10.6 and 3.06±0.05; R45: 52.5±7.4 and 3.39±0.15; R90SHAM: 58.8±5.6 and 4.00±0.98; R105SHAM: 56.7±11.1 and 2.68±0.06; R90OVX: 53.4±4.3 and 4.08±0.21; R105OVX:48.9±6.4 and 2.67±0.08; R105OVX+D0.5:38.7±5.4 and 2.05±0.12; R105OVX-D0.5: 67.3±12.3 and 2.24±0.18; R105OVX-+D0.3: 52.7±5.4 and 2.31±0.20; R105OVX-D0.3: 85.8±40.5 and 2.35±0.2. Under our experimental conditions, CTX decreased while OCN levels increased with age. Estrogen deficiency had no significant effect on bone markers, although VD-Ca insufficiency increased CTX and OCN levels considerably.

179. 98. **COMPARATIVE EFFECT OF GALACTOOLIGOSACCHARIDES (GOS) FROM TWO DIFFERENT DIETS MATRIX IN BONE HEATH OF GROWING RATS**

Mariana Seijo[1], Gabriel Bryk[1], Magali Zeni coronel M[1], Marina Soledad Bonanno[1], Claudia Vénica[2], María Luz Pita Martin de Portela[3], Claudia Bergamini[2], Irma Wolf[2], María Cristina Perotti[2], Susana Noemí Zeni[1].

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In previous studies we demonstrated the effectiveness of GOS to increase calcium (Ca) absorption and bioavailability, and to improve bone retention. Objective: to compare the effect of galactooligosaccharides (GOS) from two different food matrix on bone formation and quality during normal growth in rats. Methods: Male weaning rats were divided in 3 groups and fed until 40 days of age: AIN-93G control diet (C), GOS mixed with fructooligosaccharides (FOS) in a proportion of GOS/FOS® 9:1 (PM) or GOS mixed with probiotic bacteria's containing in an experimental yogurt diet (EY). Femur length (LF) (cm) and its Ca and phosphate (Pi) content; bone mineral density (BMD) and (BMC) of the total skeleton (tSk), lumbar spine (lSp) and proximal tibia (pT) by densitometry; bone volume percentage (BV%) and epiphyseal (GPC. Th) and hypertrophic (HpZ.Th) cartilages (μm) by histology; maximum fracture strength (N), and bone stiffness (N/mm) by biomechanical method. Shapiro-Wilk and Levene normality tests were applied, and ANOVA was performed to determine differences between groups using SPSS 19.0. p<0.05 was considering significant. Results: tSk, lSp and pT BMDs were significantly higher in EY than in PM (p<0.001) while both presented higher values than C group (p<0.010). Conversely, the highest significant tSk BMC was observed in PM (p<0.05) while EY had higher value than C (p<0.05). The highest BV and Ca and Pi content in femur was observed in PM (p<0.01 and p<0.001, respectively) while EY and C groups showed similar values. The highest significant values of GPC.Th and HpZ.Th was observed in EY (p<0.05). PM and EY showed similar values in the studied biochemical parameters than C and no significant differences between them were observed. Conclusion: Although both sources of dietary GOS induced a greater retention that benefits bone health, the symbiotic effect of GOS in the EY, due to the presence of probiotic bacteria's, appears to further enhance its effect in bone retention.

180. 250. **ANTIOXIDANT EFFECT OF RESVERATROL ADMINISTERED WITH THE CONSUMPTION OF ENRICHED WINE OR AS A DIETARY SUPPLEMENT IN PATIENTS WITH RISK FACTORS FOR METABOLIC SYNDROME**

Margarita Martínez Sarrasague[1,2], Fabiana Lairion[2,3], Alejandra Cimato[1,2,3], Raúl Pastor[4], Zulma Manfredi[4], Iris Chiesa[3], Isabel Pastor[4], Elena Pastor[4], Aldana Rodriguez[3], Christian Saporito Magriñá[2,3], Laura Iermoli[4], Carlos Bavaso[4], Claudia Taborda[5], Claudio Carbia[5], Roberto Iermoli[4], Alberto Lazarowski[5], Marisa Gabriela Repetto[2,3]

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Resveratrol (R) treatment improves the antioxidant response in cells and plasma of patients with risk factors of Metabolic Syndrome (MS) by protecting from inflammation and oxidative damage. Previous results indicated that R decreases oxygen consumption (ΔO_2) and peroxidation of lipids in PMN isolated of MS patients. The objective of this study is to compare the protective effect of R in patients with MS administered with resveratrol-enriched wine (REW) or as a dietary supplement. Voluntary patients with a diagnosis of MS (n=92) based on the diagnostic criteria of the National Cholesterol Education Program, Adult treatment Panel III, 2002 were supplemented 6 months with REW (n=30, age: 63 ± 10 years old, 150 mg/L), or 3 months with dietary R in tablets (n= 23, age: 68 ± 5 years old, 50 mg resveratrol, 25 mg alpha-tocopherol and 5 mg piperine) along with their usual treatment. Control was the patient himself in baseline conditions, avoiding interindividual variables and bioavailability of active principles. Venous blood was collected (53 patients), and biochemical markers were assessed at 0, 3 and 6 months of treatment in PMN, plasma and red blood cells: ΔO_2, lipid oxidation (TBARS), protein oxidation (carbonyl protein, CO), catalase (CAT) and superoxide dismutase (SOD) activities, and glutathione (GSH) content. At 6 months of treatment, ΔO_2 decreased with REW (56%, p<0.001). At 3 months, CO decreased 49% (p<0.01) with REW and 30% (p<0.05) with R treatments, TBARS increased 61% (p<0.05) with REW. CAT, SOD and GSH increased (30%, p<0.05; 100%, p<0.0001, and 58%, p<0.05, respectively) with R. These preliminary results indicate that R activates enzymatic and non-enzymatic intracellular antioxidant protection, reducing inflammatory processes and oxidative damage associated with MS, while REW protects against inflammatory processes and protein oxidation with the dose and treatment time evaluated.

181. 288. **ASTAXANTHIN-RICH EXTRACT DERIVED FROM CONTINENTAL NATURAL SOURCES HAS BENEFICIAL EFFECTS UPON THE ALTERED OXIDATIVE HEART STATUS AND COGNITIVE DECLINE DEVELOPED IN A DIET-INDUCED METABOLIC SYNDROME RODENT MODEL**

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