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&
XLI Meeting of the Spanish Society for Physiological Sciences

Granada, Spain

4-6 September, 2024

XXIII Meeting of the Federation of European Physiological Societies
XLI Meeting of the Spanish Society for Physiological Sciences

Granada (Spain), 4-6 September, 2024

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Sponsored by FEPS

Prof. Rodrigo Quian Quiroga – Hospital del Mar Research Institute (Spain).

PL2. CLOSING CEREMONY. Unravelling the molecular diversity of human midbrain dopaminergic neurons.
Prof. Ernest Arenas – Karolinska Institute (Sweden).

Keynote Lectures

KN1. The cellular stress response as an efficient therapeutic target for treating pancreatic cancer.
Sponsored by SECF
Prof. Juan Iovanna – Marseille Cancer Research Center (France).

KN2. Transport of negative ions over cell membranes, a positive story.
Sponsored by SPS
Prof. Christian Aalkjær – Aarhus University (Denmark).

KN3. Vascular function in cardiometabolic disorders.
Sponsored by FEPS
Dr. Elena Osto – Medical University of Graz (Austria).

KN4. Deciphering the Molecular and Secretory Functions of a Novel and Unique Brown Adipose Tissue Depot in Women.
Sponsored by Acta Physiologica "Rising Star in Physiology" Award
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KN5. Industry-sponsored lecture. Effects of 2S-Hesperidin on physical performance.
Sponsored by HTBA
Dr. Juan Ignacio Cacho Aparicio – Health Tech BioActives (Spain).

Symposia

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Sponsored by FEPS

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S2. Guanylate kinase 1 deficiency: a novel and potentially treatable mitochondrial dna depletion/deletions disease. Dr. Agustin Hidalgo – Columbia University (USA).
S3. Hypertensive pressure mechanosensing triggers transdifferentiation of vascular smooth muscle cells to foam cells. Dr. Pamela Swiatlowska – Imperial College London (UK).
S4. Cortical and hippocampal rhythmpathies in experimental models of brain metastasis evaluated with supervised learning tools. Dr. Alberto Sanchez Aguilera-Lopez – University Complutense of Madrid (Spain).
S5. Circadian rhythms in muscle stem cells. Dr. Valentina Sica – University Pompeu Fabra (Spain).

Symposium 2. IMPROVING PUBLIC UNDERSTANDING OF THE USE OF ANIMALS FOR SCIENTIFIC PURPOSES.
Sponsored by European Animal Research Association (EARA) and Spanish Society for Laboratory Animal Sciences (SECAL)

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S6. Animal research: time to talk. Mr. Kirk Leech – Executive Director EARA.
S7. Transparency in animal research in the netherlands: achievements and challenges. Dr. Monique Havermans – Coordinator Transparency Agreement on Animal Research (Netherlands).
S8. The spanish transparency agreement: an example of success. Dr. Javier Guillen – Senior Director for Europe & Latin America, AAALAC International (Spain).

Symposium 3. REGULATION OF SYNAPTIC HOMEOSTASIS IN HEALTH AND DISEASE.

Chair: Dr. Anna Fassio – University of Genoa (Italy).

S9. Dynamin-1 function in presynaptic physiology and dysfunction in epileptic encephalopathy. Dr. Mike Cousin – University of Edinburgh (UK).
S10. Role of Synapsin in Hippocampal Mossy Fiber Presynaptic Plasticity. Dr. Marta Orlando – Charité – Universitätsmedizin Berlin (Germany).

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S11. Microglial TREM2 receptor shapes CA1 metabolism and glutamatergic synapses during development. Dr. Erica Tagliatti – IRCCs Humanitas Research Hospital (Italy).

S12. The epilepsy gene TBC1D24 regulates intracellular pH homeostasis in neurons ad synapse. Dr. Anna Fassio – University of Genoa (Italy).

Symposium 4. ARE WE PROGRAMMED FOR CARDIOMETABOLIC DISEASE?.

Chair: Dr. Amanda Sferruzzi-Perri – University of Cambridge (UK); Dr Silvia Arribas – Autonomous University of Madrid (Spain).

S13. Deciphering the gasotransmitters´ function on the cardiovascular programming induced by intrauterine chronic hypoxia. Dr. Emilio A. Herrera – University of Chile (Chile).

S14. Nuclear angiotensin receptors and the fetal programming of hypertension. Dr. Carmen Diniz – University of Porto (Portugal).

S15. Effect of parental obesity on placental nutrient transport in relation to fetal developmental outcomes. Dr. Jonas Zaugg – University of Cambridge (UK).

S16. Breastmilk bioactive components to counteract fetal programming. Dr. David Ramiro-Cortijo – Autonomous University of Madrid (Spain).

Symposium 5. PHYSIOLOGY EDUCATION IN HEALTH SCIENCES.
Sponsored by the Portuguese Society of Physiology

Chair: Dr. Vicente Martínez Perea – Autonomous University of Barcelona (Spain).

S17. Current trends to improve physiology teaching and learning in the USA (ONLINE lecture). Dr. Erica Wehrwein – Michigan State University (USA).

S18. Integrating Metacognition in Physiology Education. Dr. Jorge Ascenção Oliveira – SPFis, University of Porto (Portugal).

S19. Physiology Medical Education in Health Sciences. Dr. Joaquin Garcia-Estañ – University of Murcia (Spain).

S20. Current state of Physiology (teaching and research) in the post-Bologna Europe – the case study of Portugal. Dr. Luis Monteiro Rodrigues – SPFis, University Lusofona (Portugal).

S21. The Physiology Curriculum for African Universities (PhysioCAFUN): a huge step to strengthen Physiology Education in Africa. Dr. Abdullateef I. Alagbonsi – University of Rwanda (Rwanda).

Symposium 6. ORAI CALCIUM CHANNELS: REGULATION AND FUNCTION.

Chair: Dr. Tarik Smani – Institute of Biomedicine of Seville (Spain).

S22. Activation mechanism of store-operated calcium channels: a computer simulation and live cell approach. Dr. Rainer Schindl – Medical University of Graz (Austria).

S23. Modulation of the protein content of Orai1α and Orai1β by STIM. Dr. Juan A. Rosado – University of Extremadura (Spain).

S24. Sulconazole prevents T-cell exhaustion and promotes cancer cell malignant phenotype repression by attenuation of NF-κB and calcium signaling. Dr. Isabel Galeano-Otero – University of Bordeaux (France).

S25. Role of Ca2+-sensitive adenylyl cyclase 8 in the overexpression of Orai1 after myocardial infarction. Dr. Debora Falcon – University of Seville (Spain).

Symposium 7. UNDERSTANDING THE PHYSIOLOGY OF THE BLOOD-BRAIN BARRIER: FROM MOLECULAR MECHANISMS TO MODULATION STRATEGIES.

Chair: Dr. Luca Maragliano – Polytechnic University of Marche (Italy); Dr. Valentina Castagnola – Italian Institute of Tecnology (Italy).

S26. Comparison of TJ strands architecture in epithelia and BBB endothelium. Dr. Jörg Piontek – Charité – Universitätsmedizin Berlin (Germany).

S27. Enhancing the BBB permeability using structure-based peptide design. Dr. Luca Maragliano – Polytechnic University of Marche (Italy).

S28. Paracellular and transcellular approaches to overcome the BBB. Dr. Valentina Castagnola – Italian Institute of Technology (Italy).

S29. Using the Granular Jamming Transition to 3D-Print a Blood-Brain Barrier-on-a-Chip. Dr. Joe Forth – University of Liverpool (UK).

Symposium 8. MUSCLE MITOCHONDRIA: BEYOND ATP PRODUCTION.

Chair: Prof. Jesus R Huertas – University of Granada (Spain).

S30. Electron transport chain dynamics during exercise. Dr. Rafael A Casuso – University Loyola (Spain).

S31. Skeletal muscle OXPHOS system in health and disease. Dr. Laura Formentini –

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Autonomous University of Madrid, Centre for Molecular Biology Severo Ochoa (CBM, CSIC-UAM) (Spain).

S32. Sex differences in mitochondrial functions. Dr. Sara Cogliati – Autonomous University of Madrid, Centre for Molecular Biology Severo Ochoa (CBM, CSIC-UAM) (Spain).

S33. Skeletal muscle mitochondria in extreme environments. Dr. Carsten Lundby – University of Southern Denmark (Denmark).

Symposium 9. INTEGRATIVE LEARNING: FACTORS AND APPROACHES FOR IMPROVED ACADEMIC PERFORMANCE.

Chair: Dr. Beatriz Gal – University San Pablo CEU (Spain).

S34. Relationship between sleep quality in nursing students and their academic performance: results of a randomized controlled trial. Dr. Cayetana Ruiz Zaldibar – University Camilo José Cela (Spain).

S35. Question-based learning in Physiology teaching. Prof. Dr. Cheng Hwee Ming – University of Malaya (Malaysia).

S36. Enhancing medical education through competency-based learning. Dr. Isabel Sánchez-Vera – University San Pablo CEU (Spain).

S37. Applying knowledge in context: role of integrated laboratory classes on learning physiology. Dra. Marta Lesmes Celorrio – European University of Madrid (Spain).

Symposium 10. NEW IN SILICO AND IN VITRO APPROACH METHODOLOGIES FOR DESIGNING PHYSIOLOGICALLY RELEVANT MODELS OF HUMAN TISSUES.

Chair: Prof Arti Ahluwalia – University of Pisa (Italy); Dr Elena Grasselli – University of Genoa (Italy).

S38. Lab on a Laptop. Prof. Arti Ahluwalia – University of Pisa (Italy).

S39. Skin Homeostasis and Sustainable Cosmetic Products. Dr Elena Grasselli – University of Genoa (Italy).

S40. Quantitative structure-activity relationships in pharmacokinetics: predicting human tissue permeability for drug optimization. Dr. Laureano E. Carpio – ProtoQSAR SL (Spain).

S41. The endocrine disruptor and their impact on human physiology. Dr. Giulia De Negri Atanasio – University of Genoa (Italy).

Symposium 11. EXTRACELLULAR MATRIX IN HEALTH AND DISEASE.

Chair: Prof. Eva Sykova – Institute of Neuroimmunology, Slovak Academy of Sciences Bratislava (Slovakia).

S42. Astrocytes and extracellular matrix in extrasynaptic transmission and neuroplasticity. Prof. Eva Sykova – Institute of Neuroimmunology, Slovak Academy of Sciences Bratislava (Slovakia).

S43. Dysregulation of ECM in psychiatric disorders: Impact on the connectivity of inhibitory networks. Prof. Dr. Juan Nacher – University of Valencia (Spain).

S44. The structure and distribution of the extracellular matrix affect the movement and migration of brain cells. Dr. Federico N. Soria – Achucarro Basque Center for Neuroscience (Spain).

S45. Integrity of neural extracellular matrix is required for microglia-mediated synaptic remodeling. Dr. Carla Cangalaya – German Center for Neurodegenerative Diseases (Germany).

S46.

Symposium 12. ENVIRONMENTAL FACTORS IN THE PATHOPHYSIOLOGY OF CARDIOVASCULAR DISEASES.

Chair: Prof. Devendra K. Agrawal – Western University of Health Sciences (California, USA); Prof. Dragan M. Djuric – University of Belgrade (Serbia).

S47. Pathophysiological effects of the environmental factors and treatment strategies in cardiovascular diseases. Prof. Devendra K. Agrawal – Western University of Health Sciences (California, USA).

S48. Homocysteine: a potential biomarker of environmental health risk and links to cardiovascular pathogenesis. Prof. Dragan M. Djuric – University of Belgrade (Serbia).

S49. Novel approaches in reversing environmental stress initiated pathological processes to accelerate organ repair - the power of small molecules. Prof. Ildiko Bock-Marquette – University of Pecs (Hungary).

S50. Isoprenaline model as stress induced myocardial injury. Dr. Zorislava Bajić – University of Banja Luka (Bosnia and Herzegovina)

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PLENARY and KEYNOTE LECTURES

Plenary Lectures

PL1. OPENING CEREMONY. A unique coding of memories in the human hippocampus. Prof. Rodrigo Quian Quiroga – *Hospital del Mar Research Institute, Barcelona, Spain*.

PL2. CLOSING CEREMONY. Distinct radial glia subtypes control diverse aspects of human midbrain dopaminerigc neuron development. Prof. Ernest Arenas – *Karolinska Institute, Sweden*.

Emilía Sif Ásgrímsdóttir[1], Luca Fusar Bassini[2], Pia Rivetti di Val Cervo[1,4], Daniel Gyllborg[1,5], Clàudia Puigsasllosas Pastor[1,6], Kawai Lee[1], Christopher Grigsby [1,3,7], Saiful Islam[1,8], Peter Lönneberg[1], Carlos Villaescusa[1,9], Carmen Saltó[1], Sten Linnarsson[1], Gioele La Manno[1,2], Enrique M. Toledo[1,10], **Ernest Arenas[1]**.

[1]Laboratory of Molecular Neurobiology, MBB, Karolinska Institutet, Stockholm, Sweden. [2]Laboratory of Neurodevelopmental Systems Biology, EPFL, Lausanne, Switzerland. [3]Laboratory of Medical Systems Bioengineering, MBB, Karolinska Institutet, Stockholm, Sweden. [4]Present address: Italian Medicines Agency (AIFA), Rome, Italy. [5]Present address: Dept. of Biochemistry and Biophysics, Stockholm University, Solna, Sweden. [6]Present address: Laboratory of Neural Stem Cells and Brain Damage, University of Barcelona, Spain. [7]Present address: School of Pharmacy, Queens University Belfast, UK. [8]Present address: Freenome, San Francisco, California, USA. [9]Present address: Novo Nordisk A/S, Cell Therapy R&D, Måløv, Denmark. [10]Present address: Novo Nordisk Research Centre Oxford, UK

Introduction/Objetives: Understanding midbrain dopaminergic (mDA) neuron development is key to advancing cell replacement therapies for Parkinson's disease (PD). Recent single-cell RNA-sequencing (scRNA-seq) studies have identified different subtypes of transient radial glia (Rgl) cell types in the developing mouse and human ventral midbrain. However, their individual functions and their impact on mDA neuron development are unclear.

Materials and methods: Here we use bulk and single cell RNA-sequencing to analyze the transcriptome of endogenous mouse and human ventral midbrain Rgl subtypes and to define candidate cell intrinsic and extrinsic factors controlling the development of human mDA neurons. Moreover, gain and loss of function experiments are used to assess the function of key midbrain floor plate Rgl factors in human stem cells during mDA neuron differentiation.

Results: We find that Rgl1 is defined by a neurogenic network centered on *Arntl*, and that this transcription factor regulates human mDA neurogenesis. Conversely, the transcriptome of Rgl3 is dominated by signaling and extracellular matrix molecules that control different aspects of human mDA neuron development.

Conclusion: Our results suggest a function of Rgl1 as a mDA progenitor, and of Rgl3 as a signaling mDA niche cell. Moreover, using human stem cells we demonstrate that new knowledge of cell-type specific intrinsic and extrinsic developmental factors can readily be applied to improve the generation of cells with therapeutic interest, such as human mDA neurons for PD.

Key words: Midbrain, development, dopamine, neurogenesis, Parkinson's disease, survival

Keynote Lectures

KN1. The cellular stress response as an efficient therapeutic target for treating pancreatic cancer. Prof. Juan Iovanna – *Centre de Recherche en Cancérologie de Marseille, Marseille, France*.

Introduction/Objetives: The principle of our strategy hinges on the understanding that stress induces a response enabling cancer cells to adapt and survive, thereby allowing the tumor to progress. By inhibiting this stress response, we aim to prevent cellular adaptation to stress, causing the transformed cells to die and halting tumor progression. To identify the critical stress response mechanism essential for tumor cell survival, we adopted a systematic approach in the absence of a solid rationale. Twenty-five years ago, our lab initiated a comprehensive analysis to identify genes activated in the pancreas under stress conditions. Our research revealed that the stress protein NUPR1 is significantly activated in all cancer cells, while its expression is undetectable in healthy tissues. This differential expression suggests that NUPR1 could be a promising therapeutic target for inhibiting tumor development. To validate this hypothesis, we inactivated NUPR1 in pancreatic cancer cells using a genetic approach. Our experiments demonstrated that this inactivation completely blocked tumor development in a xenograft model, serving as a proof of concept for targeting NUPR1 in cancer therapy.

Materials and methods: UPR1 is a small protein consisting of 82 amino acids and is classified as an intrinsically disordered protein (IDP). Due to its disordered structure, traditional drug development methods are not suitable for targeting NUPR1. To address this challenge, we devised an advanced screening method utilizing fluorescence thermal denaturation. Through this innovative approach, we identified an inhibitor named ZZW-115.

Results: re evaluated the anticancer activity of ZZW-115 in vitro, observing an IC50 of approximately 1 to 3 μM. In PDAC xenograft models, we found a dose-dependent antitumor effect, with the maximum effect at 5 mg/kg/day, and no apparent toxicity. NUPR1 can associate with itself or other macromolecules through its hydrophobic regions. IDPs like NUPR1 can undergo liquid-liquid phase separation (LLPS), forming large multi-protein complexes and membrane-less organelles such as stress granules. ZZW-115 impedes this agregation. To confirm NUPR1's phase separation property, we prepared solutions with increasing concentrations of NUPR1 and observed dose-dependent phase separation. By disrupting NUPR1's ability to form these complexes, ZZW-115 may interfere with pathways critical for cancer cell survival and proliferation.

Conclusion: we will present and discuss three roles of NUPR1 that can explain its antitumor effect or its effect on resistance or its role in transformation named hyperPARylation, SUMOyation and stress granules formation.

Key words: UPR1, pancreas cancer, LLPS, IDP proteins

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KN2. Transport of negative ions over cell membranes, a positive story. Prof. Christian Aalkjær – *Department of Biomedicine, Aarhus University, Aarhus, Denmark.*

Introduction/Objetives: Our insight into transport of positive ions is substantial. Our understanding of transport of the main negative ions Cl- and HCO3- is less comprehensive but has increased in recent years. It is clear that the transport of these ions are contributing to the membrane potential of many cells and is important for regulation of cell acidity. In this presentation I will provide an up to date discussion of two anion transport pathways 1) the Ca2+ activated Cl- conductance and 2) the Na,HCO3- cotransporter
Materials and methods: I will discuss the importance of these transporters in the wall of small arteries. They have been characterized in small arteries from different knock-out animals and wildtype mice. Myography, patch clamp, membrane potential recordings, ion sensitive dyes and molecular biological techniques have been used in the characterization.
Results: Two variants of the the Ca2+ activated Cl- conductance have been found - one is cGMP dependent the other is not, and their pharmacology and biophysics are different. They are both dependent on TMEM16A and the cGMP dependent variant is also dependent on bestrophins. They play a role for the electrophysiology of the vascular smooth muscle cells. The the Na,HCO3- cotransporter controls pH in the vascular smooth muscles cells and endothelial cells and is important for NO induced relaxation and for smooth muscle function.
Conclusion: We need to improve our understanding of transport of negative ions, they clearly are important for the function of tissues including blood vessels.

KN3. Vascular function in cardiometabolic disorders. Dr. Elena Osto – *Medical University of Graz, Austria*.

Background: Endothelial cell (EC) homeostasis is characterized by a quiescent and low-metabolic state. Obesity leads to EC dysfunction and CVD. We demonstrated that bariatric surgery improved obesity-induced EC dysfunction and increased fasting and post-prandial plasma levels of bile acids (BAs)(in particular, cholic acid (CA), chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA)). BAs are emerging as metabolic signaling molecules in several tissues, but their effect on ECs has not been clearly established yet. Thus, in the talk we will highlight some interest results we obtained on the capacity of BA to contribute to EC quiescence.
Methods: Human Aortic Endothelial Cells (HAECs) were treated for 24h with vehicle (0.1% DMSO), CA, CDCA and DCA (50uM each) in 2%FBS EGM-2 media. Seahorse Mitostress test, Mitoplate and glucose uptake with 0.5µCi/ml of 14C-2-deoxy-D-glucose were used to evaluate changes of HAEC metabolism. Viability and proliferation after BA treatment was checked. NO production and endothelial NO synthase (eNOS) activity were measured via DAF-2 probe and Western Blot, respectively. By qPCR genes involved in Notch pathway were evaluated. Mesoscale vascular injury panel, scratch assay, and glucose uptake were also performed in HAECs pre-treated with 50uM of BAs and stimulated with 0.5ng/mL TNF-α for 24h.
Results: HAECs treated with BAs increased NO production compared to vehicle and a reduction in both basal and maximal mitochondrial respiration and extracellular acidification rate was shown by Seahorse Mitostress test. Mitoplate assay displayed an overall reduction in the metabolic rate of HAECs treated with CA and DCA and a peculiar metabolic pattern in cells treated with CDCA. Reduction in glucose uptake was also observed after BA treatment, suggesting that BAs are able to decrease EC metabolism. Crystal Violet highlighted that BAs reduced cell proliferation. The scratch assay also displayed a reduction in EC migration after BA treatment. BAs also reduced EC apoptosis, as significant differences in the percentage of AnnexinV/PI positive cells was observed in BA-treated cells compared to vehicle. qPCR showed increased expression of genes involved in Notch pathway promoting quiescence after BA treatment. Glucose uptake, scratch assay performed in TNF-α stimulated HAECs suggested a BA-dependent endothelial protection in conditions of EC pro-inflammatory activation. We did not observe a reduction in pro-inflammatory biomarkers released in the supernatant of HAECs pre-treated with BA and stimulated with TNF-α.
Conclusions: Our results suggest that BA may be involved in the maintenance of EC quiescence by regulating EC metabolism and gene expression profile. These findings highlight a potential role of BAs as endothelial-protective molecules.
Key words: obesity, endothelial cells, bile acids, metabolism

KN4. Deciphering the Molecular and Secretory Functions of a Novel and Unique Brown Adipose Tissue Depot in Women. Dr. Borja Martínez Tellez - *Department of Nursing, Physiotherapy and Medicine and SPORT Research Group (CTS-1024), CERNEP Research Center, University of Almería, Almería, Spain. Biomedical Research Unit, Torrecárdenas University Hospital, 04009 Almería, Spain. CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Granada, Spain. Department of Medicine, Division of Endocrinology and Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands.*
Cardiometabolic diseases affect almost 50% of the population in the Western world. While lifestyle modifications and pharmacological interventions may provide short-term benefits, more research is needed to understand their long-term effectiveness and underlying molecular mechanisms. Brown adipose tissue (BAT) is a thermogenic tissue that combusts large amounts of glucose and lipids to generate heat and secretes signaling molecules known as batokines that can influence cardiometabolic health. Previous research has shown that BAT is active in adults, primarily in the supraclavicular region, as demonstrated by the uptake of 18F- Fluorodeoxyglucose. Recently, it has been demonstrated that a rare subpopulation of brown adipocytes increases in abundance at higher temperatures and can regulate the thermogenesis of neighboring adipocytes. Thus, it is plausible that classical (e.g., cold exposure) and novel interventions (i.e., Beta-2 stimulation and exercise) that activate BAT could induce a remodeling in the brown/ beige subpopulation adipocytes that govern whole tissue thermogenesis. These unstudied changes in human BAT physiology could potentially explain how BAT activation could enhance cardiometabolic health. However, despite over a decade of research, our understanding of the role of BAT in human physiology remains limited. This lack of knowledge may be mainly explained by the following: 1) obtaining biological samples of human BAT is very difficult. 2) seasonal variation strongly influences the current gold standard (PET-CT scan). Given our pilot data, young, lean adult women may possess a novel and previously undescribed thermogenic active BAT depot in the dorsocervical area (i.e., interscapular BAT or iBAT). We

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have designed a research project to discover and investigate this depot.

KN5. INDUSTRY-SPONSORED LECTURE. Effects of 2S-Hesperidin on physical performance. Dr. Juan Ignacio Cacho Aparicio – *Health Tech BioActives, Spain*.

Cacho JI[1], Cano J[1], Boza J[1], Martínez-Noguera FJ[2], Marín-Pagán C[2], Alcaraz PE[2].
[1]HealthTech BioActives S.L.U. (HTBA), Murcia, Spain. [2]Research Center for High Performance Sport, Catholic University of Murcia (UCAM), Murcia, Spain.

Flavonoids, bioactive compounds found in plants, are abundant in citrus fruits, which are one of its main dietary sources. Each citrus species contains specific flavonoids, being Hesperidin the predominant one in sweet oranges (*Citrus sinensis*). Hesperidin is well-known for its health-promoting effects, due to its antioxidant and anti-inflammatory properties. Hesperidin is a chiral molecule that exists in two isomeric forms (2S and 2 R), but only the 2S- isomer is present in nature. This natural isomerism is crucial, as it significantly impacts the properties of Hesperidin, from its bioavailability to its physiological effects.
2S-Hesperidin has been studied for its beneficial effects on cardiovascular and digestive health. Additionally, recent research has highlighted its ability to improve physical performance. Human intervention trials show that 2S-Hesperidin can increase both aerobic and anaerobic power, while extending endurance.
The mechanisms underlying this physical performance-enhancing effect include:

- Reduction of oxidative stress and inflammation, both of which are critical in mitigating fatigue and minimizing potential injuries.
- Modulation of the use of energy substrates, enhancing fat utilization during physical activity.
- Improvement of endothelial function, ensuring efficient oxygen delivery to tissues.
- Enhancement of acid-base balance and lactate clearance, which are essential for maintaining performance and delaying fatigue.
- Supporting muscle development, contributing to overall physical fitness.

2S-Hesperidin is a citrus flavonoid, that among its numerous health benefits, include the enhancement of physical performance. Available evidence highlights the potential of 2S-Hesperidin as a functional ingredient for promoting overall health and fitness and points the need for additional research on the underlying mechanism of this ergogenic effect.
Key words: flavonoids, exercise, isomerism

Symposia

Symposium 1. EARLY CAREER PHYSIOLOGISTS.
Chair: Dr. Alberto Sanchez Aguilera-Lopez – *University Complutense of Madrid, Spain*.

S1. THE PERFORMANCE-ENHANCING EFFECTS OF BLOOD DOPING AND DETECTION POSSIBILITIES IN TRAINED MALES AND FEMALES.
Andreas Breenfeldt
Research Unit for Exercise Biology, Department of Public Health, Aarhus University, Aarhus, Denmark.
Introduction: As prohibited blood manipulations such as autologous blood transfusion (ABT) and recombinant human erythropoietin (rHuEPO) injections remains a challenge for anti-doping authorities, it is important to continuously investigate and evaluate new potential detection methods and validate potential putative targets, e.g., iron biomarkers. Specifically, it is important to investigate the impact of micro-dosing strategies in relation to current detection methodologies in a sex-specific context as well as evaluate the impact of well-known confounding factors such as altitude exposure. Finally, it is important to determine the physiological effects of the applied regimes to establish the lower limit of relevance for anti-doping authorities.
Methods: The present presentation presents results from three individual studies. Study A included 48 (♀=24) well-trained participants randomized to either a placebo or a micro-dose ABT group (~130 ml of red blood cells). Study B included 48 (♀=24) well-trained participants randomized to either placebo or 9 IU/kg rHuEPO injected intravenously for four weeks. Study C included 39 (♀=16) participants receiving either rHuEPO (20 IU/kg) or placebo at altitude (2.300 m) or sea-level.
Results: In study A, a 130ml ABT where sufficient to increase hemoglobin concentration by 3.8% ($P<0.05$), while a time x treatment effect existed ($P<0.05$) for the iron regulatory markers hepcidin and erythroferrone. The Athlete Biological Passport and its sensitivity was improved when including the hepcidin and ERFE as supplementary markers. Endurance performance increased both 24 h and six days after the reinfusion (~6%, $P < 0.01$). In study B, a micro-dosing rHuEPO regimen increased total hemoglobin mass, maximal oxygen uptake and time trial performance by 6.7%, 4.2% and 4.1% (all $P<0.001$), respectively. Furthermore, immature reticulocytes seems to improve the sensitivity of the indirect detection capabilities. Finally, in study C the applied rHuEPO treatment additively increased the hematological response when administered at altitude but did also result in an additional higher chance of being detected by current detection methods.
Conclusion: Thus, even small blood doping strategies are sufficient to impact the performance of trained individuals and emphasizes the need for further research into robust and sensitive markers to detect abuse of prohibited behaviour.
Key words: Blood doping, anti-doping, athletes, erythropoietin, iron metabolism

S2. GUANYLATE KINASE 1 DEFICIENCY: A NOVEL AND POTENTIALLY TREATABLE MITOCHONDRIAL DNA DEPLETION/DELETIONS DISEASE. Dr. Agustin Hidalgo-Gutierrez[1], Jonathan Shintaku[1], Javier Ramon[2,3], Eliana Barriocanal-Casado[1], Alba Pesini[1].
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Introduction: Mitochondrial DNA (mtDNA) depletion/deletions syndrome (MDDS) comprises a group of diseases caused by primary autosomal defects of mtDNA maintenance. We have identified a new form MDDS due to biallelic GUK1 pathogenic variants in four probands presenting with ptosis, ophthalmoparesis, and myopathy, as well as variable hepatopathy and altered T-lymphocyte profile. Muscle biopsies from all probands showed mtDNA depletion or deletions, or both as well as reduced activities of mitochondrial respiratory chain enzymes. GUK1 encodes guanylate kinase (GUK1) originally identified as a cytosolic enzyme that catalyzes conversion of deoxyguanosine monophosphate (dGMP) to deoxyguanosine diphosphate (dGDP) and guanosine monophosphate (GMP) to guanosine diphosphate (GDP).
Material and methods: We have used whole exome sequencing in order to diagnose the different variants in the four probands studied. In addition, we have used a variety of molecular techniques such as bioenergetic analysis, nucleotide status, and mtDNA quantification for the functional characterization of the patients.
Results: In probands' fibroblasts, we identified decreased GUK1 activity causing unbalanced mitochondrial dNTP pools and mtDNA depletion in both replicating and quiescent fibroblasts indicating that GUK1 deficiency impairs de novo and salvage nucleotide pathways. The dual functions are consistent with our observation that the long isoform is intramitochondrial and the short isoform is cytosolic. Proband fibroblasts treated with deoxyguanosine and/or forodesine, a purine phosphatase inhibitor, ameliorated mtDNA depletion, indicating potential therapeutic interventions.
Conclussions: Our data reveal primary GUK1 deficiency as a new and potentially treatable cause of MDDS.
Key words: Mitochondrial diseases, Neuromuscular disorders.

S3. HYPERTENSIVE PRESSURE MECHANOSENSING TRIGGERS TRANSDIFFERENTIATION OF VASCULAR SMOOTH MUSCLE CELLS TO FOAM CELLS.

Dr. Pamela Swiatlowska[1,2], William Tipping[3], Emilie Marhuenda[2], Polo Severi[4], Qingzhong Xiao[2], Duncan Graham[3], Cathy Shanahan[5], Thomas Iskratsch[2].
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Introduction/Objetives: Arterial vascular smooth muscle cells (VSMCs) play central role in the onset and progression of atherosclerosis, where they undergo phenotypic switching by downregulating vSMC-specific genes and transition to a different cell type. Our previous work indicated that vSMC respond to hypertensive pressure and differential extracellular matrix compliance, exhibiting an atherosclerotic phenotype. Our aim here was to study pressure sensing in further detail.
Materials and methods: A7R5 rat vSMC were plated on glass or 1kPa polydimethylsiloxane (PDMS) and subjected to hypertensive pressure(200/120mmHg). Hyperspectral Stimulated Raman Spectroscopy (hsSRS) was applied for lipid imaging. Scratch, proliferation, TUNEL assays and RT-PCR were used to verify the foam cell phenotype. Electroporation, Western-Blot, immunocytochemistry were used to visualize Piezo1 channel. CUT&Tag analysis were applied for Piezo1-driven gene regulation changes. Lamin-associated heterochromatin was visualized with Transmission Electron Microscope. Nanoindentation was used to examine nuclear stiffness. Mouse tissue, primary rat and human vSMC were used to confirm the A7R5 findings.
Results: Following hypertensive pressure stimulation, we observed lipid droplet accumulation in vSMC as indicated by hsSRS ($p>0.05$). Similar result was obtained upon acute vSMC Piezo1, mechanosensitive channel, stimulation ($p>0.01$). Further results demonstrate upregulation of CD68, KLF4, LDLR and downregulation of ABCA1 genes ($p>0.05$), higher proliferation rate ($p>0.001$) and lower cell motility ($p>0.01$).; characteristics specific to foam cells, indicating that Piezo1 Ca2+ transients can trigger vSMC-to-foam cell transition. We identified that nuclear Piezo1 activation leads to changes in nuclear Ca2+ uptake, reactive oxygen signaling, downregulation of histone-3-lysine-9-trimethylation ($p>0.001$) and softer nucleus ($p>0.05$). CUT&Tag experiment confirmed Piezo1-epigenetic regulation of VSMC phenotypic switching.

S4. CORTICAL AND HIPPOCAMPAL RHYTHMPATHIES IN EXPERIMENTAL MODELS OF BRAIN METASTASIS EVALUATED WITH SUPERVISED LEARNING TOOLS.

Dr. Alberto Sánchez-Aguilera[1,2], Andrea Navas-Olivé[1], Mariam Masmudi-Martín[3], Patricia Baena[3], Manuel Valiente[3], Liset Menéndez de la Prida[1]
[1]Instituto Cajal. CSIC. Madrid. Spain.[2]Dpto. Fisiología. Facultad de Medicina. Universidad Complutense de Madrid. Spain.[3]Centro Nacional de Investigaciones Oncológicas. Madrid. Spain

Introduction/Objetives: Understanding the neurophysiology of brain tumours is critical to address the associated neurocognitive morbidities. Over recent years, several studies have shown alterations of neuronal network activity in peritumoral areas of primary brain tumours such as gliomas, with a bi-directional impact on cancer cells growth and on neural communication, which has been linked to hyperexcitability and seizures. However, the influence of brain metastasis on neural circuits remains relatively unexplored despite the high clinical prevalence of neurological symptoms. Here, we have evaluated microcircuit activity in the peritumoral microenvironment of mice models of brain metastasis generated by local injection of melanoma, breast and lung cancer cells.
Materials and methods: We used multi-site silicon probes in tumoral and contralateral areas of awake head-fixed mice to record the local field potential signals (LFP) from the cortex and hippocampus. Principal component analysis and different machine learning classifiers were applied to analyse electrophysiological data.
Results: Mice from melanoma, breast and lung cancer models presented a generalized reduction in the oscillatory power at different bands such as delta (1-4 Hz), theta (4-12 Hz) or ripple (100-200 Hz), and changes in the ratio between slow gamma (40-60 Hz) and fast gamma (70-90 Hz). Interestingly, we identified different LFP attenuation and tumour-type specific lateralized alterations that are not related with the size of the tumour mass. Instead, brain activity features matched with machine learning analyses confirmed model-specific alterations that could help to predict pathological electroencephalographic signals in advance.
Conclusion: Altogether, our data provide novel insights about the functional alterations associated with brain metastasis and highlight the use of predictive models for an early detection of brain metastasis signatures.
Key words: LFP, oscillations, brain metastasis, machine learning classifiers

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S5. CIRCADIAN RHYTHMS IN MUSCLE STEM CELLS. Dr. Valentina Sica[1], Jacob G Smith[1], Oleg Deryagin1 and Pura Muñoz-Cánoves[1,2]. *[1]Universitat Pompeu Fabra (UPF), Department of Medicine and Life Sciences (MELIS), 08003 Barcelona, Spain. [2]Altos Labs Inc, San Diego Institute of Science, San Diego, CA 92121, USA.*

Circadian rhythms are essential for organismal health. Satellite cells (SCs), the muscle resident stem cells, maintain a state of quiescence yet exhibit robust circadian oscillations at the transcriptional level. Although peripheral clocks have been extensively studied in various tissues, how the intrinsic clock of stem cells interacts with the central, distal clock is largely unknown. We used SC-specific reconstitution of the essential clock gene *Bmal1* to elucidate the role of the local SC clock and its interplay with the central clock in the mouse brain and found that daily transcriptional control of metabolic processes in SCs depend on central clock input, independent of the SC clock. Central clock-driven genes were involved in lipid metabolism, functionally important for SC-mediated muscle repair.

Key words: circadian rhythms, inter-organ crosstalk, stem cells, metabolism

Symposium 2. IMPROVING PUBLIC UNDERSTANDING OF THE USE OF ANIMALS FOR SCIENTIFIC PURPOSES.

Chair: Mr. Kirk Leech – *European Animal Research Association (EARA)*.

S6. ANIMAL RESEARCH: TIME TO TALK. Kirk Leech – *Executive Director European Animal Research Association (EARA).*

In a growing number of countries, public and private research institutions have made the bold decision to adopt new persuasive practices and policies to engage with the public on the benefits and achievements of using animals in scientific and biomedical research. In Europe there are now eight National Transparency Agreements on animal research in Spain, Portugal, Belgium, France, Germany, Netherlands, Switzerland and the UK involving close to 550 institutions where institutions have collectively agreed to commitments on pursuing greater openness with the public.

These commitments are that institutions; will be proactive in seeking opportunities to explain when, how and why they use animals in research; will provide information to the media and the general public about the conditions under which research using animals is carried out and will explain the benefits obtained from using them compared to other methods of research; will develop initiatives that generate greater public knowledge and understanding about the use of animals in scientific research; will place an animal welfare statement on their institution's website. The belief is that being more open and transparent about their use of animals in research will help improve public understanding and acceptance of the use of animals for scientific purposes. The need for a collective commitment is also important.

There is simultaneously growing political pressure in the USA and Europe to transition towards 'animal free science'. The research community needs to engage actively with these pressures and needs to make a stronger and clearer public case for the use of animals in research where applicable. This presentation will evaluate the experience in these countries of greater openness on the use of animals in research, and explain why we need to talk more openly about animal research.

S7. TRANSPARENCY IN ANIMAL RESEARCH IN THE NETHERLANDS: ACHIEVEMENTS AND CHALLENGES. Dr. Monique Havermans – *Coordinator Transparency Agreement on Animal Research, The Netherlands.*

At first glance, the Dutch Transparency Agreement on Animal Research (TA) is just a contract where its signatories sign up to 4 commitments. However, the TA is more than a simple extension of the legally required transparency around animal research embodied in EU directive 2010/63.

Behind the scenes, representatives of each signatory, from research and communications, form a network lead by the Stichting Informatie Dierproeven (SID), supported by the European Animal Research Association (EARA). Regular meetings allow for discussing recent developments and sharing experiences. Having both research and communications expertise from different types of organisations in the room means more constructive and fruitful discussions.

The idea of the "Open Week" is a result of these meetings. One week in the year, multiple signatories organise an event to showcase their role in animal research. Signatories are free in terms of what event they organise, according to whatever needs, possibilities, and limitations they are balancing. Examples of activities include lab tours for journalists, politicians, patients or the general public, virtual tours, explanatory videos, round-table discussions, and informal pub lectures.

One of our early learnings is the need for a coordinator, full or part time, to be able to manage the agreement. Through support of the Dutch government and administrated by SID and EARA, this has become a reality allowing for greater professionalism. Central coordination by SID gives the Agreement its moment; people stay in touch and goals stay in the foreground.

S8. THE SPANISH TRANSPARENCY AGREEMENT: AN EXAMPLE OF SUCCESS. Dr. Javier Guillen – *Senior Director for Europe & Latin America, AAALAC International, Spain.*

In 2016, the European Animal Research Association (EARA) and the Spanish Association for Laboratory Animal Science (SECAL) helped the Spanish Confederation of Scientific Societies (COSCE) promote the COSCE Transparency Agreement on the Use of Animals in Scientific Research in Spain.

It was the second to be launched after the UK's Concordat on Openness in Animal Research, and marked a pioneering effort to begin the cultural movement towards greater transparency to the public in the rest of Europe. With almost 170 signatories, the Transparency Agreement in Spain is the largest such initiative in the world.

The Spanish Agreement has clearly facilitated a cultural change (a "before and after") in the communication of animal research in Spain, with the co-ordination between the laboratory animal science community (SECAL), professionals in communication of animal research (EARA) and the research community (COSCE) proving to be essential to its success, despite being in a country lacking a specific animal research advocacy organisation. It paved the way for greater openness with the public in other countries in Europe and has grown significantly since its launch.

In this talk, genesis of the Agreement, and the practical aspects and examples of the implementation of the Agreement will be presented, to highlight their value in bringing institutions

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together, promoting knowledge-sharing and communications best practice, and ultimately shifting the landscape of animal research. The success in Spain is a testament to the impact of Agreements on facilitating cultural change on animal research in Europe and across the world.

Key words: Animal research; transparency; communication

Symposium 3. REGULATION OF SYNAPTIC HOMEOSTASIS IN HEALTH AND DISEASE.
Chair: Dr. Anna Fassio – University of Genoa, Italy.

S9. Dynamin-1 function in presynaptic physiology and dysfunction in epileptic encephalopathy. Pronot M.[1,2,3], Bonnycastle K.[1,2,3], Dobson K.L.[1,2,3], Blumrich E.-M.[1,2,3], Gajbhiye A.[4], Steinruecke M. [1,2,3], Trost M.[4], Gonzalez-Sulser A. [1,2,3], Cousin M.A. [1,2,3].
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Introduction/Objectives: The large GTPase dynamin-1 has an obligatory role in the endocytosis of synaptic vesicles (SVs) at mammalian nerve terminals. Dynamin-1 is a modular protein and numerous studies have revealed that all domains perform key roles in SV endocytosis. In recent years, pathogenic heterozygous missense mutations in the gene that encodes dynamin-1, *DNM1,* have been shown to result in a novel form of epileptic encephalopathy. Intriguingly, *DNM1* mutations cluster within regions required for its essential GTPase activity, specifically the GTPase and middle domains, implicating disruption of its enzyme activity as being central to this disorder.
Materials and methods: To determine how dynamin-1 dysfunction may result in epileptic encephalopathy, we investigated the most prevalent pathogenic mutation of *DNM1*, R237W. This was achieved via the generation of a mouse heterozygous for this allele. SV endocytosis was monitored using both optical and morphological assays, with neurotransmission assessed using patch clamp electrophysiology in hippocampal slices. Seizure-like events in vivo were monitored via electrodes recording local field potentials.
Results: Cultured neurons isolated from heterozygous *Dnm1* R237W mice displayed dysfunctional SV endocytosis. Furthermore they displayed excitatory neurotransmission and seizure-like phenotypes. Importantly, these phenotypes were all corrected via delivery of the BK channel agonist BMS-204352.
Conclusion: This work therefore establishes a direct link between dysfunctional SV endocytosis and epilepsy, and suggests that SV endocytosis is a viable therapeutic route for monogenic intractable epilepsies.
Key words: Endocytosis, vesicle, neuron, dynamin, epilepsy

S10. Role of Synapsin in Hippocampal Mossy Fiber Presynaptic Plasticity. Felicitas Bruentgens[1], Laura Moreno Velasquez[1], Alexander Stumpf[1], Daniel Parthier[1], Jörg Breustedt[1], Fabio Benfenati[2], Dragomir Milovanovic[1,3], Dietmar Schmitz[1,3], **Marta Orlando**[1].
[1]Neuroscience Research Center, Charite - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Chariteplatz 1, 10117 Berlin, Germany. [2]Center for Synaptic Neuroscience and Technology, Istituto Italiano di Technologia, 16163 Genoa, Italy. [3]German Center for Neurodegenerative Diseases (DZNE) Berlin, 10117 Berlin, Germany.

Introduction/Objetives: Hippocampal mossy fiber boutons are giant, very plastic synapses that provide a strong and sparse input onto CA3 which is important for learning and memory. These synapses have low release probability at rest but display various form of presynaptic plasticity: frequenty facilitation, post-tetanic potentiation (PTP), and cAMP-dependent long-term potentiation (LTP).
Synapsins are the most abundant neuronal phosphoproteins. Due to their phosphorilation-dependent association in dimers and tetramers and with synaptic vesicles they regulate vesicle clustering and availability, playing an important role in synaptic transmission and plasticity.
Differently form most synapses, hippocampal mossy fiber boutons retain synapsin III expression in the mature brain. We sought to determine if the knockout of all synapsin isoforms would influence mossy fiber boutons' structure and function.
Materials and methods: To this aim we combined transmission electron microscopy and local field potential recordings of acute hippocampal slices from synaptin triple knockout (SynTKO) and wild type (WT) male mice.
Results: We observed that both frequency facilitation and the PTP after high-frequency stimulation were lower in SynTKO animals, in line with previous experiments from synapsin double knock-out animals. We also measured LTP and saw that SynTKO mossy fibers displayed an increased potentiation 30 minutes after induction accompanied by increase in AT density at the ultrastructural level.
Conclusion: The impairment in short term plasticity in SynTKO mossy fibers is likely a consequence of the smaller reserve pool of vesicles observed at the electron microscope. The increase in LTP observed here in SynTKO was not detected in Syn DKO or single synapsin knockouts: we speculate that it could be due to the absence of syn III. Further experiments investigating mossy fiber plasticity at synapsin III knockout mice will help us define whether this specific isoform plays a role in presynaptic potentiation at hippocampal mossy fiber boutons.
Key words: mossy fibers, synaptic plasticity

S11. Microglial TREM2 receptor shapes CA1 metabolism and glutamatergic synapses during development. **Erica Tagliatti**[1,2], Genni Desiato[1], Sara Mancinelli[3], Matteo Bizzotto[1,3], Maria Cristina Gagliani[4], Rebeca Hernandez-Soto[1], Andrea Cugurra[1], Paola Poliseno[1], Faggiani Elisa[1], Matteo Miotto[3], Fabia Filipello[1], Arguelllo Raphael[5], Katia Cortese[4], Raffaella Morini[1], Simona Lodato[3,1] and Michela Matteoli [1,6].
[1]IRCCS Humanitas Research Hospital, Milan, Italy; [2]University College London, London, UK; [3]Humanitas University, Department of Biomedical Sciences, Milan, Italy; [4]Università di Genova, Genova, Italy; [5]Aix Marseille Univ, CNRS, INSERM, CIML, Marseille, France; [6]National Research Council, Milan, Italy.
Introduction/Objetives: A continuous crosstalk between microglia, the brain residential immune cells, and neurons is required for the maintenance of brain homeostasis and for the sculpting of neuronal connections during development. The Triggering Receptor Expressed on Myeloid cells 2 (TREM2),

a immunoglobulin-like receptor exclusively expressed by microglia in the brain, is central in shaping this crosstalk. During brain development, TREM2 plays a critical role by directly controlling the process of synapse elimination. How Trem2 signaling impacts neuronal development and synaptic maturation, however, is still an unresolved question.
Materials and methods: By using a multidiplinary approach, we characterized how the lack of TREM2 receptor in microglia impacts on neuronal maturation. We perfomed scRNA sequencing analysis to detect any transcriptional changes in the neuronal populations of the hippocampus at postnatal day 1. We next employed confocal microscopy, electron microscopy and metabolic measurements to assess neuronal metabolic profiles throughout brain development. Ultimately, we used electrophysiology and live imaging tools to measure neuronal maturation and synaptic function.
Results: In the absence of Trem2, developing neurons in the hippocampus display compromised energetic metabolism, which is paralleled by a significant transcriptional rearrangement of hippocampal pyramidal neurons at birth and a delay in the maturation of CA1 neurons. Notably, these early developmental alterations impact on neuronal activity and social behaviour in young adult mice.
In addition, after isolation from the brain context, hippocampal neurons mantain a faulty maturation, suggesting that an early TREM2-depedent microglia to neuron crosstalk is sufficient to derange the forthcoming maturation of neurons.
Conclusion: Our results unveil a novel role of TREM2 in controlling neuronal development and synaptic function by regulating the metabolic fitness of neurons in a region-specific manner.
Key words: TREM2, Microglia, Mitochondria, Synapse

S12. The epilepsy gene TBC1D24 regulates intracellular pH homeostasis in neurons ad synapse. Anna Fassio[1,2], Sara Pepe[2], Chiara Penzo[1], Simone Giubbolini[1], Hanako Tsushima Semini[1].
[1]Departement of Experimental Medicine, University of Genoa, Genoa Italy. [2]IRCCS, Ospedale Policlinico San Martino, Genoa Italy.

Introduction/Objetives: The acidity of intracellular compartments is fundamental to favor the different physiological functions along the endocytic and degradative pathways. In neurons, in addition to the endo-lysosomal acidic compartments, synaptic vesicles (SVs) are continuously acidified to allow the efficient regeneration of neurotransmitter quanta. The vacuolar ATPase (v-ATPase) is responsible for hydrogen ion transport into intracellular organelles and in neurons it is highly expressed at SV membrane. V-ATPase function is finely regulated from yeast to humans; however, little is known about neuronal and synaptic controllers of v-ATPase activity.
Materials and methods: We employed hippocampal and cortical primary neurons from newly developed Tbc1d24 null mouse model and perform immunocytochemistry, live imaging, ultrastructural analysis and fractionation experiments to measure intra-organellar acidification, SV cycling, synapse morphology and autophagy progression. We also employed IPSC-derived neurons for evaluation of neuronal development and electrophysiological activity in control and patient conditions.
Results: Here we describe a novel role for TBC1D24 as a neuronal regulator of v-ATPase association state and activity. We revealed that TBC1D24 interacts with v-ATPase in the brain. Using a newly developed Tbc1d24 null mouse model, we observed accumulation of lysosomes and lipid materials in the brain. In Tbc1d24 null neurons, we detected a increased dissociation of the v-ATPase complex alongside increased pH at intracellular compartments and autophagy impairment. At Tbc1d24 null synapses, we revealed defective SV reacidification, with reduction of SVs and accumulation of cisternae-like structures.
Conclusion: The data demonstrate that TBC1D24 is a regulator of v-ATPase in neurons and synapses and that dysregulation of pH homeostasis could be at the basis of neurodevelopmental and epilepsy disorders associated with *TBC1D24* and *v-ATPase* mutations.
Key words: synaptic vesicle, lysosome, pH, autophagy, neurodevelopmental disorders, TBC1D24, knockout,

Symposium 4. ARE WE PROGRAMMED FOR CARDIOMETABOLIC DISEASE?.
Chair: Dr Amanda Sferruzzi-Perri – University of Cambridge (UK); Dr Silvia Arribas – Autonomous University of Madrid (Spain).

S13. Deciphering the gasotransmitters´ function on the cardiovascular programming induced by intrauterine chronic hypoxia. Emilio A Herrera[1,2], Adolfo A Paz[1], Tamara A Jiménez[1], Felipe Beñaldo[1], Pedro Herrera[1], Alejandro González-Candia[3].
[1]Vascular Function & Reactivity Laboratory, Faculty of Medicine, Universidad de Chile, Santiago, Chile. [2]International Center for Andean Studies (INCAS); Universidad de Chile, Putre, Chile. [3]Institute of Health Sciences, Universidad de O'Higgins, Rancagua, Chile.

Introduction/Objetives: Chronic hypoxia during gestation (CH) increases susceptibility to cardiovascular (CV) diseases during postnatal life, associated to increased oxidative stress (OS), CV remodeling and dysfunction. Furthermore, CH impairs gasotransmitters (CO, NO and H2S) function; however, the effects on its synthesizing enzymes have not been studied in the CV system. We aimed to study the effects of prenatal hypoxia and melatonin as antioxidant treatment in the cardiovascular function and gasotransmitter-synthesizing enzymes regulation.
Materials and methods: 24 Pregnant guinea pigs were pseudo-randomly divided in 4 groups: normoxia (720 torr, N), hypobaric hypoxia (470 torr, H), N melatonin treated (NM) and H melatonin treated (HM). Melatonin was given 1mg/kg/day orallly to the pregnant dams until term. Fetal cardiac structure and function was determined by echocardiography during gestation. After delivery, all groups offspring was raised under standard normoxic conditions, followed by ultrasound doppler and euthanized at juvenile (n=6 per group) or adulthood (n=6 per group) for ex vivo (wire myography, vascular function) and in vitro (expression of vasodilators and gasotransmitters) analyses in heart, aorta, carotid and femoral arteries. All procedures were approved by the Local Bioethical Committee (CICUA 20354-MED-UCH).
Results: Hypoxia and melatonin alters the structure and function of the analysed cardiovascular tissue, and this was associated with the hemoxigenase gasotransmitter (CO) expression modifications and its gene methylation.
Conclusion: Chronic hypoxia during gestation induces cardiovascular changes at different stages of life associated with HO gene epigenetic modifications.
Funded by Fondecyt n°1201283 & 1241502.
Key words: Developmental programming, hypobaric hypoxia, pregnancy, cardiovascular, endothelial function

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S14. Nuclear angiotensin receptors and the fetal programming of hypertension. Dr. Carmen Diniz – *LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal*

Introduction: Cardiovascular disease (CVD) is the leading cause of noncommunicable disease morbidity/mortality worldwide. When adverse intrauterine conditions occur, changes in fetal development have been identified to ensure survival, leading to increased susceptibility to CVD and hypertension later in life. The predisposition to fetal programming of hypertension (FPH) has been linked to changes in the renin-angiotensin system (RAS) and its interaction with the sympathetic nervous system (SNS). RAS receptors (AT1, AT2, Mas, MrgD) are G-protein coupled receptors (GPCRs). The intracellular localisation of GPCRs has been described and respective actions have been identified, namely cell proliferation, angiogenesis and survival, as well as their involvement in disease processes such as CVD including FPH.
Material and Methods: FPH animal model; Isolation of Nuclei/Plasma Membranes from FPH tissues; Immunohistochemistry; Measurement of NO/ROS using fluorescent probes; Evaluation of sympathetic activity using transmitter release experiments (3H-Noradrenaline).
Results: We have identified that the induction of hypertension by fetal nutrient restriction is associated with morphological changes in the vessels, namely an increased expression of AT1 receptors, ACE and ACE2 as well as NADPH oxidase and conversely a reduction of RAS receptors (AT2, Mas and MrgD). Also, a sympathetic hyperinnervation in the outer layer of the vessels has been identified, together with an inward remodelling. These morphological changes are accompanied by hyperactivation of sympathetic neurotransmission, at least partly related to a tonic facilitation (by endogenous Ang II) through AT1 receptors, and also to an increase in oxidative stress as evidenced by plasma oxidative damage biomarkers, increase in ROS and NADPH activity. Interestingly, nuclear AT1 and AT2 receptors are implicated in signalling pathways leading to increased oxidative stress (as a result of reduced NO availability and increased ROS) and increased collagen synthesis and fibroblast proliferation (via IP3).
Conclusion: Nuclear angiotensin receptors exert important effects contributing to oxidative stress and vascular hypertrophy in FPH.
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S15. Effect of parental obesity on placental nutrient transport in relation to fetal developmental outcomes. Jonas Zaugg[1], Samantha C Lean[1], Megan Vickers1, Alejandro A Candia[2], Edina Horvath-Gulacsi1, Amanda N Sferruzzi-Perri[1].
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The rate of maternal obesity is increasing in the UK, affecting over one-fifth of pregnancies, primarily due to diets high in carbohydrates and fats. Maternal obesity increases the risk of fetal growth abnormalities and can programme metabolic defects in offspring, e.g. type-2 diabetes, obesity, and cardiovascular disease. Iron, an essential micronutrient, is unidirectionally transported from the mother to the fetus by the placenta and is crucial for liver development and fetal growth. However, excess iron triggers ferroptosis (iron-dependent cell death) with impacts on growth and physiology.
We aimed to investigate how maternal obesity affects iron handling and ferroptotic pathways in the placenta and fetal liver using a mouse model of pre-gestational maternal obesity induced by a high-fat/high-sugar diet. Results showed that maternal blood iron and tissue stores during pregnancy were unchanged in obese compared to lean mice. However, fetal serum iron levels and the ratio of maternal to fetal blood iron concentration, an estimate of placental transport capacity, were reduced in obese pregnant mice. Maternal diet-induced obesity decreased iron deposition and oxidative stress in the placental labyrinth zone (Lz), responsible for materno-fetal nutrient transport. It also reduced the expression of heme-iron transporters and anti-ferroptosis markers in the Lz, while increasing the expression of iron transporters, regulators like hepcidin, and the pro-ferroptosis marker *Acsl4*. There were also changes in iron homeostasis genes in the fetal liver, including iron receptor *Tfr1* and multiple ferroptosis markers, due to maternal obesity. Placentas, fetuses, and fetal livers were significantly lighter in obese pregnancies. However, postnatally, offspring of obese mothers exhibited catch-up growth, and achieved a normal body weight by 12 weeks of age, although their liver size remained reduced. Furthermore, there were programmed alterations in the expression of key iron regulatory genes in the offspring liver, indicating altered ferroptosis management with elevated iron uptake.
These findings illustrate that maternal obesity disrupts placental iron handling, activates ferroptotic pathways, and leads to mal-development of the fetal liver and placenta. Although postnatal growth normalized, there were lasting changes in the expression of genes crucial for iron homeostasis in the liver of offspring from obese mothers. These results are concerning as obesity reaches epidemic proportions globally.
Key words: Maternal obesity, placental iron handling

S16. Breastmilk bioactive components to counteract fetal programming. Dr. David Ramiro-Cortijo – *Autonomous University of Madrid, Spain.*

The intrauterine environment is modulated by different gestational factors being nutritional access to the fetus relevant for its development, known as fetal programming. Birth weight is a relevant variable implicated, not only in neonatal survival, but also in future cardiometabolic diseases (CMD) development. In singleton gestations, increased CMD risk is associated with both high and low birth weight (LBW). Prematurity, linked LBW, is a pandemic with high impact on future CMD development, being facilitated by postnatal growth acceleration (catch-up growth). While this phenomenon is an advantage for neonatal immune response and neurodevelopment, it is also a contributing factor for type 2 diabetes, hypertension and overweight development. Infant nutritional strategy by breastmilk (BM) can counteract fetal programming, through an adequate macronutrient level, providing physiological growth, and bioactive components implicated in organ maturation. BM has antioxidants, hormones, cytokines and long-chain polyunsaturated fatty acids (LC-PUFAs), precursors of specialized pro-resolving mediators (SPMs), which could protect against catch-up growth and reduce CMD risk. Some bioactive components are synthesized by the mammary gland, while others derive from

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adipose tissue or her diet, being influenced by maternal health and habits.
An important research question is how BM bioactive components can impact premature infant health to counteract fetal programming. Colostrum has the highest antioxidant levels decreasing along lactation and are higher in women with preterm labor. Interestingly, these factors are lacking in formulas, and decreased in donated milk due to thermo-preservation processes. Prematurity down-regulates some appetite-related hormones, which modulate neonatal growth, being influenced by maternal body composition and diet. However, the role of LC-PUFAs and derived SPMs remain controversial. While BM provides the LC-PUFASs precursors, the SPMs are synthesized by the neonate based on tissular maturation, leading to different outcomes.
In conclusion, BM provides bioactive factors which can help to counteract fetal programming, being relevant to monitor maternal diet and body composition, particularly in women with preterm birth. Since these women are not always capable to provide complete breastfeed, it would be important to develop more physiological fortifiers matching BM bioactive components. The science of BM opens a new therapeutic option to minimize the health consequences exploring infant nutritional programming.
Key words: Catch-up growth; Breastmilk; Hormones; Antioxidants; SPMs; Nutritional programming.

Symposium 5. PHYSIOLOGY EDUCATION IN HEALTH SCIENCES.

Sponsored by Portuguese Society of Physiology (SPFIS)
Chair: Dr. Vicente Martínez Perea – *Autonomous University of Barcelona, Spain.*

S17. Current trends to improve physiology teaching and learning in the USA. Dr. Erica Wehrwein – *Michigan State University, USA.*

Introduction/Objectives: There is a growing interest globally in the teaching and learning of physiology at all levels of training from primary school through medical school. In the USA, there are major efforts underway over the past 6 years that are focused on building community among physiology undergraduate degree programs and educators, emphasizing the importance of teaching from the "core concepts" of physiology, focus on building student professional skills, and improving physiology instructor readiness in the classroom.
Materials and methods: Using the data and objectives of the Physiology Majors Interest Group (P-MIG) and the American Physiological Society's Center for Physiology Education (CPE), the presentation will share current trends in Physiology education in the USA with emphasis on professional skills development, integrative Physiology teaching, and core concepts of physiology.
Results: There is a consensus among the key players in US physiology education that professional skills development and core concepts teaching are the current best practice for excellent teaching and Learning. There are also efforts underway to improve the teaching of medical school physiology and integrating into the evolving curriculum.
Conclusion: The coordinated efforts of P-MIG and APS CPE have been fruitful in building consensus among physiology educators for how to beat teach in our discipline.
Key words: physiology education, teaching, learning

S18. Integrating Metacognition in Physiology Education. Dr. Jorge Ascenção Oliveira – *SPFis, University of Porto, Portugal.*

Introduction/Objectives: The learning of physiology provides ample opportunity for cognitive development. To understand the mechanisms of human body function, one should go beyond simple associative memory, and establish a dynamic mental network of cause-effect relationships. Establishing such mental network requires metacognition - the awareness and understanding of thought processes – to accurately self-assess, monitor, and improve by self-regulation. Our objective was to explicitly integrate metacognitive development in the physiological education strategy.
Materials and methods: Pharmaceutical sciences students from the University of Porto self-assessed their interest in physiology and their study strategies (Survey-1). They then monitored their learning in weekly formative tests, including self-assessment (score prediction), and received counselling on active study techniques. Next, they self-assessed their study adaptations (Survey-2), and were rewarded for accurate score predictions in Exams. Anonymised data from tests and surveys were analysed with R for statistics and machine learning (ML).
Results: Unsupervised ML of Survey-1 identified three student clusters with different levels of self-reported interest in physiology and study investment, which associated with different Exam scores. A supervised ML model trained with data from formative tests predicted Exam failures with over 90% accuracy, and the actual Exam scores (0-20) with error under 2 points. Analysis of formative tests over time evidenced significant improvements in students' metacognitive ability to accurately self-assess, despite being challenged with questions about different physiological systems over time. Moreover, the frequent report of study adaptations in Survey-2 supports widespread metacognitive regulation.
Conclusion: This study provides evidence-based support for enhanced student metacognition and suggests that ML models can accurately predict student failure, enabling early counselling. To promote physiological skills, we emphasise the need for authentic assessment. By integrating metacognitive development, we aim to empower students and foster a continuous improvement mindset.
Key words: metacognition; self-assessment; active study

S19. Physiology Medical Education in Health Sciences. Dr. Joaquin Garcia-Estañ – *University of Murcia, Spain.*

Introduction: We have made an analysis of main changes in physiology teaching in medical education in Spain during the last years. Medical education in Spain has undergone significant changes over the years. These changes are driven by advancements in medical science, educational methodologies, and the need to adapt to evolving healthcare demands. Traditionally, medical education in Spain separated basic sciences (like physiology) from clinical training. Modern curricula are increasingly integrated, allowing students to apply physiological concepts directly to clinical scenarios.
Methods: This is not happening in our study area due to several reasons most of them shared by other basic topics such as biochemistry, cell biology or anatomy. We will analyze some of them.
Results: Most of the professors now teaching medical physiology are not medical doctors and this difficults the integration in a clinically-oriented curricula. Another important reason is the lack of importance of physiology in the

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medicine title as perceived by medical students. This is observed when medical students see that the MIR exam that every doctor should pass to obtain access to medical specialization only has 2 or 3 physiology questions out of more than 200 to answer. It is difficult to tackle these problems, but the future physiology professors should reflect on how to increase the love of their students for the physiological processes.
Conclusion: High quality standards and innovation, in the field of teaching and scientific research should be the basis for a cultural change among medical students, followed by an intent to increase integration with the clinical matters, for instance by increasing the amount of Pathophysiology at the clinical years and giving a clinical perspective to all the Physiology processes. Integration of physiology and other basic topics in the clinical subjects is also another objective to improve the situation of physiology in medical education. An integrated curriculum can be a fundamental change to be performed by the future physiology educators.
Key words: Innovation, medical education, integrative physiology

S20. Current state of Physiology (teaching and research) in the post-Bologna Europe – the case study of Portugal. Dr. Luis Monteiro Rodrigues, João Gregório.
Universidade Lusófona | CBIOS -Research Center for Biosciences & Health Technologies, Lisbon, Portugal.

Introduction/Objetives: The European High Education Area (EHEA) is an ongoing project with multiple challenges. The Bologna process was a major harmonisation instrument within the European Union, although with multiple impacts, both expected and unexpected, still requiring deeper analysis. In that direction, this study provides a current overview of Physiology teaching and research in post-Bologna Portugal.
Materials and methods: Physiology teaching data publicly available was collected from the National Higher Education Directorate agency (DGES) from September to November 2022. This allowed us to design a descriptive study to analyse Physiology teaching in the higher education institutions and health-related syllabi. Furthermore, identifiable lecturers' scientific production between 2017-2022 was also screened using Web of Science and PUBMED databases.
Results: A total of 365 courses/degrees and 764 Physiology disciplines were identified related with health-related professions, mostly administered in universities and technical colleges. However, universities and technical colleges differ greatly in programs, staff experience and scientific profiles. University medical schools were found to provide the most complete formation in human physiology teaching and research, while technical colleges provided the highest number of physiology related disciplines. The hands-on training is also prevalent in universities. Regarding research and faculty differentiation, university and technical colleges also greatly differ. Staff with relevant production are affiliated with reference university centers or research units.
Conclusion: In order to define a coherent and viable identity for European physiology and physiologists, a better knowledge and understanding of the state of Physiology in the EHEA is needed. Special focus on differences in teaching and research modalities can provide a basis for comparison and support the development of an appropriate path for physiology as a discipline.
Key words: education; EHEA; post-Bologna Process

S21. The Physiology Curriculum for African Universities (PhysioCAFUN): a huge step to strengthen Physiology Education in Africa. Dr. Abdullateef I. Alagbonsi – *University of Rwanda, Rwanda.*

Background: Physiology education in Africa has weaknesses associated with deficiencies in the contents of curricula used across many African Universities, with divergent contents and competencies lacking benchmarking. Thus, it is difficult to have comparable learning outcomes in Physiology teaching, and professionalizing physiology is a mirage. For the first time, we developed the Physiology Curriculum for African Universities (PhysioCAFUN) as a first step to address these problems.
Materials and Methods: We constituted a committee of 15 Physiologists from East Africa (Rwanda, Tanzania), Southern Africa (South Africa, Zambia, Namibia), North Africa (Egypt, Sudan), West Africa (Nigeria, Senegal), Germany, and USA to draft the PhysioCAFUN. The draft was presented during the joint conference of the East African Society of Physiological Sciences (EASPS) and African Association of Physiological Sciences (AAPS) held in Tanzania late 2023. Inputs from the conference were considered and the approved version was published on the AAPS website.
Results: The PhysioCAFUN has 23 modules. Modules 1-15 cover organ systems, including principles and concepts of Physiology, molecular biology and cell physiology, cell-cell communication and control systems, integrated control of fluid-electrolyte and acid-base homeostasis, cellular neurophysiology, systems neurophysiology, and the physiology of endocrine, muscle, blood, immune, cardiovascular, respiratory, renal, reproductive, gastrointestinal and metabolic systems. Modules 16-23 cover optional contents, including environmental physiology, pharmacology, principles of animal experimentation and biomedical instrumentation, epidemiology biostatistics and research methodology, clinical placement in laboratories, seminars on current topics in physiology, physiology entrepreneurship, and research project.
Conclusion: PhysioCAFUN serves as a freely-available resource document for African stakeholders regarding the desired undergraduate physiology training and competencies. It will help universities in some African nations to freshly draft a curriculum suitable for their local needs where there is dearth of physiologists, or to benchmark and revise their curricula where physiology programs are already in place. We are collaborating with physiological societies across Africa to work with universities and stakeholders in their territories and persuade them for the gradual implementation of PhysioCAFUN.
Key words: Africa; Competencies; Curriculum; Physiology

Symposium 6. ORAI CALCIUM CHANNELS: REGULATION AND FUNCTION.
Chair Dr. Tarik Smani – *Institute of Biomedicine of Seville Spain*.

S22. Activation mechanism of store-operated calcium channels: a computer simulation and live cell approach. Matthias Sallinger[1], Christina Humer[1], Hwei Ling Ong[2], Sasirekha Narayanasamy[2], Qi Tong Lin[3], Marc Fahrner[1], Herwig Grabmayr[1], Sascha Berlansky[1], Sean Choi[2], Tony Schmidt[4], Lena Maltan[1], Lara Atzgerstorfer[1], Martin Niederwieser[4], Irene Frischauf[1], Christoph Romanin[1], Peter B. Stathopulos[3], Indu Ambudkar[2], Romana Leitner[1], Daniel Bonhenry[5] and **Rainer Schindl**[4]

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Introduction/Objetives: The single-pass transmembrane protein Stromal Interaction Molecule 1 (STIM1), located in the endoplasmic reticulum (ER) membrane, has two primary functions: sensing ER- Ca^{2+} concentration and directly binding to the store-operated Ca^{2+} channel Orai1 to activate it when Ca^{2+} levels decrease.
Materials and methods: Here, we present our findings using a combined approach of molecular dynamics (MD) simulations, purified proteins, and live cell techniques.
Results: When the ER-Ca2+ concentration is high, the ER-luminal STIM1 domain remains monomeric. However, once Ca^{2+} stores are depleted, STIM1 undergoes di-/multimerization. This multimerization is crucial for exposing the C-terminal binding site of STIM1, which interacts with Orai1 channels. Mutations that destabilize the resting configuration of STIM1 can lead to tubular aggregate myopathy. Despite its importance, the structural basis of the luminal association sites has been elusive. We identified two critical di-/multimerization segments: the α7 helix and the adjacent region near the α9 helix in the sterile alpha motif (SAM) domain.
Conclusion: I will discuss how interactions between the SAM domains of STIM1 monomers are vital for the protein's multimerization and activation.
Key words: store-operated channels, calcium signaling

S23. Modulation of the protein content of Orai1α and Orai1β by STIM. Dr. Juan A. Rosado[1], Joel Nieto-Felipe[1], Alvaro Macias-Díaz[1], Vanesa Jimenez-Velarde[1], Jose J. Lopez[1], Gines M. Salido[1], Tarik Smani[2], Isaac Jardin[1].

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Introduction/Objetives: The endoplasmic reticulum (ER)-sensing proteins STIM1 and STIM2 activate CRAC channels during store-operated Ca^{2+} entry (SOCE), where Orai1 plays a major role. Two Orai1 variants have been identified in mammalian cells. Orai1α, comprises 301 amino acids, while the short form, Orai1β, is generated by alternative translation initiation at either methionine 64 or 71 in the Orai1α variant. We have investigated the role of STIM proteins in the modulation of Orai1α and Orai1β expression.
Materials and methods: CRISPR-generated STIM1/2 double-knockout HEK293 cells (DKO) cells were transfected with Orai1α or Orai1β in combination with STIM1 or STIM2. Orai1-STIM interaction was analyzed by confocal Förster Resonance Energy Transfer and immunoprecipitation.
Results: DKO cells transfection with Orai1 variants in combination with STIM1 or STIM2 led to a significant drop in Orai1α and Orai1β expression both at the Orai:STIM ratio of 1:1 and 1:2, being significantly smaller at the 1:2 ratio. Co-transfection of DKO cells with Orai1 variants in combination with the constitutively active mutants STIM1-D76A and STIM2-D80A resulted in a greater inhibition of Orai1 protein expression. We discerned between a role for STIM proteins in the synthesis rate and the stability of Orai1 by using the synthesis inhibitor cycloheximide (CHX). In the presence of CHX STIM expression reduced the expression of Orai1 variants, an effect that was prevented in the presence of bafilomycin A1, an inhibitor of the vacuolar H^+-ATPase that prevents lysosomal protein degradation, by contrast, treatment with MG132, which impairs proteasomal degradation, did not alter the modulation of Orai1 expression by STIM.
Conclusion: These findings indicate that STIM proteins play a relevant role in Orai1α and Orai1β recycling by lysosomal degradation.
Key words: Orai1α and Orai1β expression, STIM proteins, lysosomal degradation.
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S24. Sulconazole prevents T-cell exhaustion and promotes cancer cell malignant phenotype repression by attenuation of NF-κB and calcium signaling. Dr. Isabel Galeano-Otero – *University of Bordeaux, France.*

Introduction/Objectives: The overexpression of the immunoinhibitory receptor programmed death-1 (PD1) on T-cells plays a significant role in cancer immune evasion. While anti-PD-1/PDL-1 therapies have revolutionized cancer treatment and improved patient survival, their efficacy varies widely across different tumor types and patient populations. Consequently, novel treatments are needed to interfere with the anti-tumoral immune responses and propose an adjunct therapy.
Methods: In this study, we explored the impact of the antifungal drug Sulconazole (SCZ) on PD-1 expression in activated PBMCs and T cells at both RNA and protein levels. Furthermore, we investigated SCZ's effects on NF-κB and calcium signaling pathways. Additionally, we assessed SCZ's influence on cancer cell proliferation, migration, and tumor growth using zebrafish embryo models. The drug's ability to inhibit calcium mobilization in cancer cells was also evaluated.
Results: Our results indicate that SCZ effectively inhibits PD-1 expression on activated PBMCs and T cells at both the RNA and protein levels. SCZ suppressed NF-κB and calcium signaling, crucial pathways involved in PD-1 induction. Furthermore, SCZ treatment significantly reduced cancer cell proliferation, migration, and tumor growth in vitro and in zebrafish embryos. SCZ also demonstrated an ability to inhibit calcium mobilization within cancer cells.
Conclusion: These findings highlight the potential of SCZ as a therapeutic agent, either used alone or in combination with existing treatments, to prevent T-cell exhaustion and suppress the malignant phenotype of cancer cells. This dual approach could enhance tumor eradication and improve overall cancer treatment outcomes.

S25. Role of Ca2+-sensitive adenylyl cyclase 8 in the overexpression of Orai1 after myocardial infarction.
Dr. Débora Falcón[1,2]**,** Eva Calderón[1,2], Isabel Mayoral[1], Marta Martín[1,2], Antonio Ordóñez[1], Juan Antonio Rosado[3] and Tarik Smani[1,2].

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of Medical Physiology and Biophysics, Faculty of Medicine, University of Seville, Seville, Spain. [3]Department of physiology, Institute of Molecular Pathology Biomarkers (IMPB), University of Extremadura, Cáceres, Spain

Introduction/Objetives: The upregulation of Orai1 and subsequent store-operated Ca^{2+} entry (SOCE) has been associated with adverse cardiac remodelling and heart failure (HF). However, the mechanism driving Orai1 upregulation and its involvement in myocardial infarction remains unclear. The **objective** of this study was to examine the molecular mechanism and signalling pathways involved in Orai1 upregulation after ischemia/reperfusion (I/R) in heart.
Materials and methods: Experiments were performed in adult (ARVM) and neonatal rat ventricular cardiomyocytes (NRVM), in a rat model of myocardial I/R, and in ventricular biopsies of patients with ischemic heart failure. Ca^{2+} studies were realized in FURA-2AM loaded cardiomyocytes. Immunofluorescence and Western Blot was used to investigate CREB, Orai1 and adenylyl cyclase 8 (AC8) protein expression.
Results: We observed that I/R increases the expression of Orai1 and Ca^{2+}-sensitive AC8 in rats' heart, which was associated with reduced ventricular contractions and an increase in the diastolic intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$). Similarly, we found significant overexpression of Orai1 and AC8 in ventricular biopsies of post-ischemic HF patients. Mechanistically, I/R-induced activation of Orai1 stimulates AC8, which generates cAMP and consequently induces CREB phosphorylation. p-CREB, in turn, activates Orai1 promoter and facilitates I/R-induced Orai1 upregulation. The intramyocardial administration of AAV9 carrying sh-RNA of AC8 reduces the expression of AC8 and Orai1, restores diastolic $[Ca^{2+}]_i$ and recovered significantly cardiac contraction.
Conclusion: Our results show that the activation of Orai1/AC8/CREB axis plays a key role in the I/R-induced Orai1 upregulation, which participates in diastolic $[Ca^{2+}]i$ mishandling related to the adverse cardiac remodelling.
Key words: SOCE, ischemia/reperfusion, Orai1, calcium

Symposium 7. UNDERSTANDING THE PHYSIOLOGY OF THE BLOOD-BRAIN BARRIER: FROM MOLECULAR MECHANISMS TO MODULATION STRATEGIES.

Chair: Dr. Luca Maragliano – *Polytechnic University of Marche, Italy*; Dr. Valentina Castagnola – *Italian Institute of Tecnology, Italy*.

S26. Comparison of TJ strands architecture in epithelia and BBB endothelium.

Dr. Jörg Piontek – *Charité – Universitätsmedizin Berlin, Germany.*

Introduction/Objetives: The paracellular transport in epithelia and endothelia is regulated by tight junctions (TJ). The backbone of TJ strands/fibrils is formed by claudins. The composition and consequently the barrier properties of TJs differ strongly between different tissues. The very strong solute barrier of the blood brain barrier (BBB) is mainly determined by claudin-5, however, the underlaying molecular mechanism is largely unclear.
Materials and methods: Confocal, STED and freeze fracture microscopy with different epithelial and brain endothelial cell lines and primary cells were used to analyze and to compare the claudin composition of the respective TJs. Variants of the claudin-binding domain of the Clostridium perfringens enterotoxin (cCPE) were used to detect claudins or to modulate the TJ barrier. Molecular modeling and molecular dynamics (MD) simulations were used to generate models of the claudin-based TJ strand architecture.
Results: Freeze fracture EM still provides the highest resolution to visualize the unique ultrastructure of BBB TJs. However, with STED microscopy the protein composition of TJ strands and associated complexes can be better identified. In contrast to epithelial in vitro models of many different tissues, in vitro BBB models recapitulate the in vivo situation much worse due to dedifferentiation. Comparison of different in vitro BBB models suggest that claudin-5 is the main barrier-forming component of BBB TJs. A clear contribution of Cldn3, -11, -12 could not be observed. In models based on human-induced pluripotent stem cells (hiPSCs) multiple claudins could be identified in the TJ, namely at least CLDN4, -5, -6 suggesting that several claudin subtypes can copolymerize into the barrier-forming TJ strand meshwork of BBB-like cells. However, care has to be taken, since the observed claudin composition could be also the result of a differentiation into cells with a mix endothelial and epithelial phenotype. In addition, cCPE-based molecular tools targeting claudins for confocal or STED detection of barrier opening were generated. Furthermore, sequence analysis and MD simulations suggest that endothelial TJs consisting mainly of Cldn5 and epithelial TJ consisting mainly of other classic claudins share a similar overall TJ strand architecture.
Key words: tight junction, claudins, modeling, microscopy

S27. Enhancing the BBB permeability using structure-based peptide design.

Dr. Luca Maragliano – *Polytechnic University of Marche, Italy.*

Introduction/Objetives: the blood-brain barrier (BBB) is essential to maintain brain homeostasis and healthy conditions, but it also prevents drugs from reaching brain cells. BBB endothelial cells are joined together by tight junctions (TJs), multi-protein complexes that regulate paracellular diffusion and whose major component is claudin-5 (CLDN5), playing a pivotal role in restricting the paracellular traffic. In view of obtaining fine control over the transport across the BBB, the use of competing peptides able to bind CLDN5 to induce transient and regulated permeabilization of the paracellular passage is emerging as a potentially translatable strategy for clinical applications. We designed and tested short peptides with improved solubility and biocompatibility using a combined approach that involved structural modeling techniques and in vitro validation, generating a robust workflow for the design, screening, and optimization of peptides for the modulation of the BBB paracellular permeability.
Materials and methods: the performed molecular modeling, atomistic Molecular Dynamics simulations, microscale thermophoresis, mass spectrometry, immunofluorescence, TEER measurements and transport studies.
Results: The computational analysis classified all tested peptides based on solubility and affinity to CLDN5, and provided atom-level details of the binding process. From our screening, we identified a novel CLDN5-derived peptide that demonstrated good solubility in biological media, efficient binding to CLDN5 subunits, and capability to increase permeability at low concentrations.
Conclusion: the set up a robust in silico/experimental workflow for the design and validation of competitive peptides to open CLDN5 multimeric assemblies in the BBB TJs. Our platform, which includes complementary validation of peptide

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solubility, binding affinity, and paracellular space opening, can be widely applied to screen potential short peptides with a potential affinity for any TJ protein.
Key words: light junctions, molecular dynamics, TEER, microscale termophoresis

S28. Paracellular and transcellular approaches to overcome the BBB. Dr. V. Castagnola[1,2], S. Vercellino[1,2], M. Trevisani[1], V. Cartocci[1,2], L. Ulfo[1,2], A. Berselli[1], G. Alberini[1], L. Boselli[1], H. Ahmed[1], L. Maragliano[3], F. Benfenati[1,2].
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Introduction: Reaching the brain for non-invasive therapeutic interventions still represents a formidable challenge for our society. One of the main difficulties is related to the presence of the blood-brain barrier (BBB), an essential protection mechanism that selectively limits the passage of molecules into the delicate neuronal environment but, on the other hand, significantly hinders the effective delivery of therapeutic formulations administered via the bloodstream. Nanomaterials, given their propensity to engage with biological machinery actively, open opportunities to exploit transcellular routes to overcome the BBB. However, for nanoformulations traveling in the bloodstream, the formation of a biomolecular corona masking the engineered materials' identity significantly limits this approach. Complementary approaches involve the modulation of the tight-junction binding between adjacent cells to increase the BBB permeability. In this work, we propose novel strategies to exploit both paracellular and transcellular routes across the BBB, also in a synergic fashion.
Materials and methods: In this work, we employed in vitro models of BBB of increasing complexity, from transwell monolayer systems to co-cultures and 3D multicellular assemblies, to assess the efficacy of biological carriers in overcoming the endothelial layer and delivering molecules to a selected subpopulation of neurons. The same models were exploited to assess barrier permeabilization. Tight junctions-binding peptides were designed for this purpose with the help of molecular modeling.
Results: We exploited a biomimetic approach to avoid coronation and enhance the BBB crossing ability. We selected M13 bacteriophages, harmless bacteria-specific viruses largely present in our body, as a safe and effective vehicle. Phages offer facile genetic manipulation, enabling the expression of targeting moieties on their capsids, and are produced through a scalable process. We show how these flexible nanocarriers, by eluding the protein corona and keeping their integrity and engineered tropism, can be used for transport across BBB. In a complementary strategy, using competitive binding peptides designed with an in-silico approach, we were able to loosen the binding of adjacent tight-junction proteins, making the paracellular space more permeable to small molecules.
Conclusion: Altogether, our approaches offer exciting prospects for advancing therapeutic interventions in neurological disorders.
Key words: BBB, hybrid nano constructs, tight junctions

S29. Using the Granular Jamming Transition to 3D-Print a Blood-Brain Barrier-on-a-Chip. Dr. Joe Forth – *University of Liverpool, UK.*

Introduction/Objectives: The blood-brain-barrier (BBB) tightly regulates the transport of material between the bloodstream and the brain. Designing drugs that can cross this barrier is a formidable challenge. A significant obstacle to progress here is the lack of high-fidelity BBB models that recapitulate the transport mechanisms by which pharmaceutical compounds can enter the brain. Here, I discuss how jammed microgel suspensions can be used as biocompatible suspension baths to produce a vascularised BBB model.
Materials and Methods: A custom-built bioprinter was derived from a Prusa Mini+ with gcode for microvascular patterning produced using a bespoke non-planar algorithm for omnidirectional direct ink writing. A biocompatible suspension bath was prepared from gelatine methacryloyl that was processed into jammed cross-linked microgels via batch emulsification. Microgels were further immersed in a chemically identical, non-crosslinked GelMA filler phase that yielded optically transparent suspension baths. Microvascular patterns were written into the suspension baths using Pluronic F-127 sacrificial inks. The sacrificials inks were removed after printing and the suspension bath was subsequently cross-linked. Human brain microvascular endothelial cells and human primary astrocytes were seeded on the gels.
Results: GelMA was processed into microgel suspensions with a particle diameter of 10 - 200 um, with particle size characterised using microscopy and laser diffraction particle size analysis. Shear rheology showed that at GelMA mass fractions in the range 6-10% w/w, these suspensions had yield-stress/shear-thinning rheology with tunable elastic modulus in the range 10 - 10000 Pa, comparable to in-vivo values. These rheological properties in-turn allow the microgel suspensions to act as support baths for the printing of complex patterns of branched vasculature with diameter ranging from 100 - 1000 um. Humary primary astrocytes and immortalised hCMEC/D3 brain endothelial cells could be seeded onto these materials at high viabilities.
Conclusion: An approach to fabricating vascularised tissue scaffolds suitable for BBB modelling has been demonstrated. Our approach allows for highly flexible formulation of the suspension bath, which further acts as a biocompatible, synthetic extracellular matrix (ECM). Preliminary cell work demonstrates the viability of both brain microvascular endothelial cells and astrocytes on the materials, showing a promising avenue for producing a complex BBB model comprised of physiologically relevant materials and microvascular structure.
Key words: Blood-Brain-Barrier; Vasculature; Bioprinting, Tissue Engineering

Symposium 8. MUSCLE MITOCHONDRIA: BEYOND ATP PRODUCTION.

Chair: Prof. Jesus R Huertas – *University of Granada, Spain*; Dr. Rafael A Casuso – *University of Loyola, Spain*.

S30. Electron transport chain dynamics during exercise. Dr. Rafael A Casuso[1,2] and Jesús R. Huertas[2].
[1]University Loyola (Spain); [2]University of Granada (Spain).

Skeletal muscle plays a pivotal role in whole body metabolism. Furthermore, skeletal muscle mitochondrial volume density can significantly increase in response to exercise within human muscle cells, likely reflecting the high oxygen demands required by exercised muscle. We have previously documented that high-intensity exercise induced morphological changes that connect oxygen-rich areas at the sarcolemma with the core of the muscle cell, wich has limited access to oxygen. Exercise training can also induce changes in the supramolecular

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organization of the electron transport chain, where the respiratory complexes are assembled into higher-order structures named supercomplexes (SCs). While some scientific groups believe that these SCs are merely structural entities, growing data suggest that they are functional entities, for instance, through modulating reactive oxygen species production. In fact, dysfunction in proper SC assembly can significantly reduce exercise capacity. The specific assembly between the complexes I, III and IV can indeed be modulated to accomodate electron chanelling for the available substrate. Our latest data show that brief high intensity exercise can modulate the assembly of SCs within skeletal muscle. This observation can help not only to elucidate the functionality of SCs but also to understand the molecular mechanisms underlying the healthy effects of exercise.

Key words: Exercise, mitochondria, muscle

S31. Skeletal muscle OXPHOS system in health and disease. Beñat Salegi Ansa[1], José María Lanza Arnaiz[1], Tzoliné Topdjian Panosian[1], **Laura Formentini** [1,2,3].
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[2]*Instituto Universitario de Biología Molecular, IUBM, Universidad Autónoma de Madrid (UAM), Madrid, Spain.*
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Introduction/Objetives: Coenzyme Q (Q) is a key lipid electron transporter involved in a variety of essential biological processes and in maintaining mitochondrial homeostasis. However, several aspects of its biosynthesis and redox biology remain undefined. Many different mitochondrial flavoproteins reduce the Q; however, in eukaryotes, only the OXPHOS-complex III (CIII) oxidizes the QH2. The mechanism of action of CIII is still debated. We aim at clarifying it.
Materials and methods: We have developed a skeletal muscle-specific mouse model for the deletion of ETFDH, a mitochondrial Q reductase that channels electrons from lipid catabolism to CIII. Using various in vivo and in vitro approaches (immunoprecipitations and BN-PAGE gels coupled with proteomics, enzymatic activities in isolated skeletal muscle mitochondria, mathematical models, etc.), we aimed to understand the dependency of CIII activity on ETFDH
Results: Herein, we demonstrate that ETFDH is essential for CIII activity in skeletal muscle. We identify a complex involving ETFDH, CIII, and the Q-biosynthesis regulator COQ2 that directs electrons from lipid substrates to the respiratory chain, reducing electron leak and ROS production. This metabolon maintains Q levels, minimizes QH2-reductive stress, and improves OXPHOS efficiency. Muscle-specific ETFDH-/- mice develop myopathy due to CIII dysfunction.
Conclusion: In skeletal muscle, ETFDH is essential as an OXPHOS component and represents a potential therapeutic target for mitochondrial redox medicine.
Key words: bioenergetics, coenzyme Q, ETFDH, mitochondria, OXPHOS.

S32. Sex differences in mitochondrial functions. Dr. Sara Cogliati – *Autonomous University of Madrid, Centre for Molecular Biology Severo Ochoa (CBM, CSIC-UAM), Spain.*

Introduction/Objetives: Historically, women have been largely excluded from clinical trials, resulting in a deficit of sex-specific medical knowledge and an erroneous assumption that findings derived from male subjects apply equally to females. Recent studies have shed light on the "sexosome," which reflects sex-biased effects on gene networks and cellular systems. However, the underlying molecular mechanisms remain largely unknown. Thus, understanding sex differences at the molecular level is vital for the advancement of personalized medicine. Biological differences between sexes are governed by sex chromosomes and hormones, both of which can influence mitochondrial functions. Among various tissues, the liver and heart exhibit significant sexual dimorphism, notably influenced by estrogen. However, the molecular connection between mitochondrial function and liver physiology within the context of varying estrogen levels remains unexplored.
Materials and methods: We analyzed mitochondrial functions in the liver and heart of male mice, fertile female mice, and ovariectomized female mice. All animals were sacrificed at 14-16 weeks of age. The fertile female mice were further divided into high and low estrogen groups based on their proestrus and diestrus phases. The estrous phase was monitored daily using the vaginal smear technique. Ovariectomies were performed on 12-week-old female mice to remove their ovaries.
Results: Our results indicate that the absence of estrogen leads to alterations in mitochondrial morphology, the organization of the respiratory chain, and the activity of ATP synthase
Conclusion: We have demonstrated that estrogen significantly impacts mitochondrial functions and contributes to the observed mitochondrial sex differences.
Key words: mitochondria, sex-differences, sex-hormones.

S33. Skeletal muscle mitochondria in extreme environments. Dr. Carsten Lundby.
Department of Sports Science and Clinical Biomechanics, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark.

A key function of skeletal muscle mitochondria is the transduction of energy for muscle contraction. This depends on a consistent supply of oxygen and since high-altitude exposure causes a decrease in oxygen availability, high-altitude sojourn may therefore act as a stimulus on skeletal muscle mitochondria.
Under conditions of preserved body weight, unchanged muscle fiber area, maintained physical activity level and access to palatable food, exposing sea-level residents to high altitude for weeks generally increases muscular mitochondrial volume density (MitoVD). Nonetheless, an increase in muscular MitoVD does not necessarily imply an increased mitochondrial respiratory capacity. Mitochondrial respiratory capacity has been evaluated using high-resolution respirometry on skeletal muscle samples both before and after acclimatisation to high altitude. Studies show trivial effects on maximal coupled respiration normalised to overall tissue sample mass (i.e. mass-specific) when Caucasians are exposed to high altitude for weeks to months. However, to accurately assess the respiratory capacity of the mitochondrial content, and its possible qualitative intrinsic adaptations, it is necessary to combine mass-specific respiratory measures with quantitative measures defining skeletal muscle mitochondrial content. Here, the structural measure muscular MitoVD reflecting the skeletal muscle mitochondrial volume has typically been used as well as commonly used biomarkers hereof such as citrate synthase activity. Accordingly, a ~30% decrease in maximal coupled respiration normalised to citrate synthase activity after about one month at high altitude.

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However, most of the complexes of the electron transport system (ETS) and the adenosine triphosphate (ATP) synthase complex are localised in the cristae membrane. Consequently, the ATP-producing capability of mitochondria should inherently be dependent on the cristae surface area per mitochondrial volume referred to as mitochondrial cristae density (MitoCD). Accordingly, respiration per cristae surface area may provide a more accurate evaluation of mitochondrial intrinsic capabilities than respiration per mitochondrial volume. The aim of this presentation is to highlight the effect of high-altitude exposure on mitochondrial respiratory capacity and intrinsic capabilities by combining MitoCD with mass-specific mitochondrial respiration.

Key words: Mitochondrial volume density, mitochondrial cristae density, mitochondrial respiration, high altitude.

Symposium 9. INTEGRATIVE LEARNING: FACTORS AND APPROACHES FOR IMPROVED ACADEMIC PERFORMANCE.

Chair: Dr. Beatriz Gal – *University San Pablo CEU, Spain.*

S34. Relationship between sleep quality in nursing students and their academic performance: results of a randomized controlled trial.

Dr. Cayetana Ruiz-Zaldibar[1,2], Beatriz Gal-Iglesias[3], Clara Azpeleta-Noriega[4], David Pérez-Manchón[1,2]

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Introduction/Objetives: University students are especially vulnerable due to sleep disruptors such as the intensity and dedication to study and its impact on the emotional level. This has been related to poorer academic performance, behavioral and psychological problems.

The aim of this study is to evaluate the impact of an intervention aimed at improving sleep quality in nursing students and its effect on their academic performance. A pilot randomized controlled trial was conducted with 40 participants (intervention group (IG)=20; Control group (CG)=20) at a private university in Madrid. The IG received two 90-minute sessions during two weeks on sleep physiology, habits, and sleep hygiene.

Materials and methods: Sleep variables was performed with the Kronowise 3.0 wristband device (Kronohealth, S.L., Spain) for 7 consecutive days under normal living conditions, before the intervention and 10 days afterwards in both groups. This device is validated against polysomnography and generates 23,000,000 raw data in 7d to provide 14 primary variables on circadian and sleep parameters based on thermometry, acceleration, position and light exposure. The Pittsburgh Sleep Quality Index (PSQI) and sociodemographic variables, BMI, toxic habits and physical exercise were measured. For academic performance, the Attention and Concentration test d2 was used before and after the intervention and grades in the physiology subject.

Results: The IG increased the student`s stability and inter-day regularity of circadian rhythm (p=0.042) comparing with CG. The greatest effect was observed in the main circadian health marker (CHS) improving the IG versus CG (p=0.025). Regarding sleep quality, the IG improved in indirect factors such as greater exposure to white light 2h and less exposure to blue light 2h before sleep and direct factors such as lower sleep latency. Insomnia was substantially associated with poor academic performance in female nursing students. Positive trend related to their academic performance were found for IG.

Conclusion: This study evidences the positive effects of a health promotion intervention to improve the sleep quality of college students and its effects on academic performance.

Key words: Sleepquality; University students; Academic performance

S35. Question-based learning in Physiology teaching.

Dr. Cheng Hwee Ming – *University of Malaya, Malaysia.*

The enquiry approach to learning is effective across ages. Children learn and remember through repetitive question and answer. In the dynamic, integrated multi-organ physiology, there are homeostatic concepts and diverse mechanisms to comprehend.

The use of focussed questions can highlight key perspectives in physiological processes. In the large class setting, the use of questions to break up the lecture helps engage the student audience. Withholding from giving immediate answers to questions posed can encourage curiosity that propel self-directed learning.

Several examples of questions formulated to address common misconceptions will be illustrated.

When students are encouraged to comfortably ask questions, they enjoy homeostatic learning. And educators who carefully design physiology questions also benefit from the time spent in simplifying explanations to clarify the complex, beautiful whole body homeostasis.

Cheng HM, Hoe SZ(2021) The WRITE Physiology: Thinking and Inking. BLDE Univ J Health Sciences 6:90

Key words: Questions, Concepts, Curiosity, Engagement

S36. Enhancing medical education through competency-based learning.

Dr. Isabel Sánchez-Vera, Antonio Sobrado de Vicente-Tutor, Esther Escudero, María del Nogal, Úrsula Muñoz, María José Borrego, Hortensia Torres, Josué García, Cruz Sádaba.

Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad CEU San Pablo, CEU Universities, Madrid, Spain.

Introduction/Objetives: Beyond the acquisition of technical and methodological knowledge, it is equally critical to develop what are known as transversal competencie. These include the hability to be flexible and adapt to different situations, to work in a team, to think critically, to make decisions and to be reflective, analytical, respectful, empathetic and supportive. These competencies are essential for the effective application of knowledge in real-life situations and underscores the need to implement learning strategies that develop these types of skills. Our aim was to design a competency-based learning plan and an assessment system that would allow us to adequately measure the level of competency-based progress acquired during physiology course.

Materials and methods: We used active methodologies based on simulation environments and role playing during the Physiology practical and theoretical classes in the Medicine Degree second year students. Initially we developed protocol that structures the acquisition of practical clinical skills for students while reinforcing the theoretical concepts taught in class. Besides, we presented a rubric specifically designed and implemented for the evaluation of this specific and transversal competencies. Additionally, we introduced a rubric

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specifically designed to evaluate both specific and transversal competencies during the physiology practical sessions. Furthermore, we employed group dynamics as a didactic methodology to foster the development of competencies such as decision-making, collaborative teamwork, and the exchange of ideas

Results: Students put into practice the knowledge acquired in different clinical situations and worked on the communication and treatment of the patient. The practical learning approach in pairs and constant feedback from the professor promote the development of technical skills and the improvement of doctor-patient communication. Data analysis included academic outcomes, and student perceptions through surveys. Results indicated that competency-based learning has a positiveimpact on students' skills and academic performance. Participants reported high levels of satisfaction and found these activities enriching and motivating. In addition, students who participated scored higher on the final course assessment than those who did not participate.

Conclusion: The development of competency-based learning strategies is essential for acquiring competencies in a medical degree program.

Key words: competency-based learning, rubrics, role-playing

S37. Applying knowledge in context: role of integrated laboratory classes on learning physiology. Dr. Marta Lesmes Celorrio – *European University of Madrid, Spain*.

Introduction/Objetives: Physiology content is perceived by students as rather difficult, who may lack vision on how to relate it with their professional training. Therefore, identifying novel active and more engaging pedagogical strategies for teaching physiology to psychology students may help to fill this gap. In this pilot study, we used the PBL methodology developed around a clinical case to evaluate psychology students' experience and learning in two laboratory classes modalities. The aim of this study was to compare undergraduates' preference for laboratory classes taught either independently (cohort 1, $n = 87$ students) or integrated into the PBL-oriented clinical case (cohort 2, $n = 92$ students) for which laboratory classes were transformed into Integrated Laboratory Classes (ILCs). The students' academic performance was also evaluated to look for quantitative differences between cohorts

Materials and methods: In this pilot study, we aimed to assess the impact of academic performance and student's experience of teaching laboratory classes contextualized within the PBL applied to Physiology teaching in first year psychology degree. Two cohorts from consecutive academic years were selected: cohort 1 ($n = 87$) from 2018/19, which was considered the control group, and cohort ($n = 92$) during 2019/2020 as the experimental group. Cohort 1 was organized in three homogeneous group of students each of 25–30 students. Cohort 2 was organized in four heterogeneous group of students, two of them composed by 30 students and the other two of lessthan 30 students. For quantitative analysis, academic scores from each of the different blocks in both cohorts were compared using an inferentialnon-parametric statistical study. Additonaly a questionnaire to assess student´s experience was distributed.

Results: When we compared the academic scores obtained in the theoretical content from each cohort, we found a significant improvement ($p < 0.05$) in cohort 2 where the students achieved better results as compared to cohort 1. A subset of students was asked to fill a questionnaire assessment on their experience and found that 78.9% of them preferred integrated laboratory classes over laboratory classes alone. They consistently reported a better understandingof the theoretical content and the value they gave to ILCs for learning.

Conclusion: In conclusion, our pilot study suggests that integrating laboratory classes into PBL-oriented clinical contexts help to retain core physiology contents and it can be considered as an engaging learning activity worth implementing in Psychology teaching.

Key words: integrated learning, PBL methodology, laboratory classes.

Symposium 10. NEW IN SILICO AND IN VITRO APPROACH METHODOLOGIES FOR DESIGNING PHYSIOLOGICALLY RELEVANT MODELS OF HUMAN TISSUES.

Chair: Prof Arti Ahluwalia – *University of Pisa, Italy*; Dr Elena Grasselli – *University of Genoa, Italy*.

S38. Lab on a Laptop. Botte E[1], Mancini P[1], Fontana F[1], Magliaro C[1,2], **Ahluwalia A**[1,2].
[1]*Centro di Ricerca E. Piaggio, University of Pisa, Italy.* [2]*Centro 3R, Italy.*

Introduction/Objectives: Human physiology and pathology are generally investigated using in-vivo or animal models or in vitro systems based on cell cultures. While animal models are expensive and may not represent human organs and systems entirely faithfully, cell-based in vitro models are often too simplistic. In silico models can provide crucial support to both, enhancing experimental design and data interpretation in animal experiments and supporting the development of more complex in-vitro models involving fluidics and 3D cultures.

Materials and methods: I will first introduce the COST Action IMPROVE, a network focused on the underlining the relationship between 3Rs practice and better-quality science. Then, I will discuss in silico models and show how they can be used to improve experimental outcomes, in line with the 3Rs (Replacements, Refinement, Reduction of animal experimentation) principles.

Results: Finally, I will present innovative computational and artifical intellegence based methods which integrate genetic algorithms, statistical mechanics, allometric scaling and finite element simulation of individual and collective cellular resource (mainly oxygen) consumption.

Conclusion: These "lab-on-a-laptop" models could, in the future, augment or even substitute in vivo and in vitro models providing a more sustainable, high-fidelity way of conducting experiments, particularly if our computers and laptops are powered by renewable energy sources.

Key words: in silico models, 3Rs, cell oxygen metabolism

S39. Skin Homeostasis and Sustainable Cosmetic Products. Elena Grasselli[1,2], G. Allaria[1], L. Dondero[1], G. De Negri Atanasio[1], M. Passalacqua[2,3], E. Lertora[1], F. Rispo[1], F. Tardanico[1], R., S. Ferrando[1], I. Demori[4]. [1]*Department of Earth, Environmental, and Life Sciences (DISTAV), University of Genoa, 16132 Genoa, Italy.* [2]*Interuniversity Center for the Promotion of 3R Principles in Teaching and Research (Centro 3R), Italy.* [3]*Department of experimental medicine (DIMES), University of Genoa, 16132 Genoa, Italy.* [4]*Department of Pharmacy (DIFAR), University of Genoa, 16132 Genoa, Italy.*

Introduction/Objetives: The skin, as the largest organ of the body, serves to protect living organisms by separating the external environment from the internal, thereby maintaining all

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essential life functions. Cosmetic products are designed to interact with the all the body surface, including skin, primarily for cleaning, perfuming, altering appearance, correcting body odors, and protecting or maintaining skin health. Recently, there has been a trend towards using cosmetic ingredients derived from otherwise wasted resources. This approach supports the WHO's 2030 Agenda for sustainable development and aligns with the One Health concept, which highlights the interconnection among nature, wildlife, and human beings. One aim of the H2020 EcoeFISHent project is to establish a circular value chain for cosmetics in the Liguria region of Italy.

Materials and methods: By EU law, testing of cosmetic products must avoid the use of animal models. To this aim, human skin models were conceived: Reconstructed Human Epidermis (RHE), which recapitulates all the skin strata, consisting of organized and proliferative basal cells, spinous and granular layers, and cornified epidermal layers, and are mitotically and metabolically active.

Using this model, we tested both the safety, in terms of skin physiology maintenance, and the beneficial effects of certain active principles, as well as formulated cosmetic products derived from sea sidestreams, specifically fish oils and hydrolyzed collagen.

Results and Conclusion: Using new approach methodologies (NAMs), we assessed the beneficial effects of both fish oils and hydrolyzed collagen. In particular, fish oils were able to prevent oxidative stress in the epidermis, while hydrolyzed collagen improved the dermis by promoting collagen deposition and increasing water content. These results demonstrate that the sustainable use of sidestreams can be a starting point for promoting the responsible use of natural resources.

Key words: skin physiology, reconstructed human epidermis, new approach methodologies, Sustainable use of sidestreams

S40. Quantitative structure-activity relationships in pharmacokinetics: predicting human tissue permeability for drug optimization. Dr. Laureano E. Carpio – *ProtoQSAR SL, Spain.*

Introduction/Objectives: A critical challenge in the drug development process is the poor permeability profiles of many promising compounds, which often leads to their failure. Quantitative structure-activity relationships (QSAR) applied in pharmacokinetics offer a potential solution by predicting human tissue permeability using *in silico* models. This study explores the application of QSAR models to forecast human tissue permeability, focusing on key parameters such as blood-brain barrier permeation and intestinal absorption. By employing advanced computational techniques, we aim to enhance drug optimization, streamline the drug development process, reduce experimental costs, and improve the efficiency of identifying viable drug candidates with optimal permeability profiles.

Materials and methods: Initially, we sourced data from publicly available pharmacokinetic and chemical databases, obtaining a set of compounds with documented permeability characteristics. The collected data underwent preprocessing to address missing values, and ensure consistency across the dataset. Relevant molecular descriptors were calculated and selected using statistical methods to identify the features most predictive of tissue permeability. Subsequently, we employed machine learning algorithms to train the QSAR models, evaluating various models, including linear regression, decision trees, and neural networks, to identify the most accurate predictors. The models were validated using cross-validation techniques and an independent test set to assess their predictive accuracy and robustness Finally, the QSAR models were integrated in the module ProtoADME of the ProtoPRED server.

Results: Different QSAR models with optimal metrics were successfully developed. These models exhibited predictive accuracy and robustness, effectively forecasting permeability profiles for a range of compounds. These models were integrated in an online platform that allowed us to perform different predictions of compounds.

Conclusion: In conclusion, the models demonstrated reliable predictions for critical parameters such as blood-brain barrier permeation and intestinal absorption. These results underscore the models' potential to enhance drug development and streamline the identification of viable drug candidates.

Key words: QSAR, ADME, Tissue permeability, pharmacokinetics.

S41. The endocrine disruptor and their impact on human physiology. **Dr. Giulia De Negri Atanasio**[1], Francesca Rispo[1], Lorenzo Dondero[1], Giorgia Allaria[1], Francesca Tardanico[1], Erica Lertora[1,2], Elisabetta Perata[2], Jan Markus[3], Silvia Letasiova[3], Alessandro Alberto Casazza[4], Matteo Zanotti Russo[2], Elena Grasselli[1,5].

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Introduction/Objetives: Increasing scientific concern exists about the nature and the safety of the ingredients used by the cosmetics industry regarding their endocrine-disrupting effects. Due to their daily use, the presence of an endocrine disruptor (ED) in a cosmetic can strongly impact human health. A "cosmetic product" shall mean any substance or mixture intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition. People use an average of seven different products per day, demonstrating that cosmetics are used substantially by everyone.

Moreover ED present in daily use product could also have an impact in the eviroment such as the two class of filters which are actually used in cosmetic formulation un sunscreen.

Materials and methods: In the simulated exposure scenario, the samples were applied onto the 3D reconstructed skin models (EpiDerm) followed by evaluation of viability using MTT assay at different concentrations for 24h and 48h. Quantitative RT-PCR was performed to analyze the effects of genistein and daidzein on the mRNA expression of estrogen receptors (ERs). Molecular docking calculations were performed to elucidate the binding modes of the compounds with ER alpha and beta.

Results: The samples showed no significant harmful effects in tissue viability. Gene expression of ER alpha and ER beta were differently affected by different concentrations and time points.

Conclusion: We have designed a tiered approach to evaluate effects of potential ED that involves exposure of a target organ

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(skin) and evaluation of multiple parameters including viability, regeneration and gene expression. In the future we plan to incorporate a reporter system capable to detect endocrine pathways activation.
Key words: Enviroment, cosmetics, endocrine disruptors, percutaneous permeation

Symposium 11. EXTRACELLULAR MATRIX IN HEALTH AND DISEASE.
Chair: Prof. Eva Sykova – *Institute of Neuroimmunology, Slovak Academy of Sciences Bratislava, Slovakia*.

S42. Astrocytes and extracellular matrix in extrasynaptic transmission and neuroplasticity. Dr. Eva Sykova.
Institute of Neuroimmunology, Slovak Academy of Sciences Bratislava (Slovakia).

Introduction: The extracellular matrix (ECM) is a network of macromolecules secreted by neurons and glia. The ECM modulates synapse formation, neuroplasticity, neural cell migration, differentiation, axonal growth, sprouting, and pathfinding. It has two forms, diffuse ECM (dECM) and perineuronal nets (PNNs). Both forms of ECM influence brain development, aging, brain pathologies and CNS plasticity. Furthermore, ECM significantly influences extrasynaptic (volume) transmission, which is mediated by diffusion of neuroactive substances in the extracellular space (ECS) (for rev. see Sykova and Nicholson, Phys. Rev. 2008).
Material and methods: We studied the changes in astrocytes, PNNs and dECM during aging and brain pathologies. For temporal disruption of ECM rats were treated with 4-methylumbelliferone (4-MU), an inhibitor of hyaluronan (HA) synthesis. We used the RTI method (real-time iontophoretic method using ion-selective microelectrodes) in rat cortex to study the effects of disruption of the ECM on the ECS diffusion parameters. Noninvasive DW-MRI was used to show changes in mean diffusivity (MD) and fractional anisotropy (FA).
Results: Disruption of PNNs and dECM occurred during aging, after brain or spinal cord injury, in models of Alzheimer's disease and of Amyotrophic Lateral Sclerosis. The changes were accompanied by functional deficits and modified by stem cell application or manipulation of ECM. The oral treatment of rats with 4-MU disrupted PNNs, decreased staining for chondroitin sulphate proteoglycans (CSPG), HA and downregulated staining for astrocytes. A 35 % increase in ECS volume fraction was found by RTI method, similar to the value found in rat cortex during early postnatal development. In DW-MRI studies we found a decrease in MD and loss of FA.
Conclusions: We conclude that the observed increase in ECS volume, loss of FA and downregulation of astrocytes evoked by dysregulation of PNNs and dECM can affect extrasynaptic transmission, cell to cell communication and neural plasticity. Supported by APVV-17-0642 and APVV-230436.
Key words: astrocytes, extrasynaptic transmission, neurodegenerative diseases, plasticity

S43. Dysregulation of ECM in psychiatric disorders: Impact on the connectivity of inhibitory networks. Dr. Juan Nacher – *University of Valencia, Spain.*

Introduction/Objetives: The perineuronal nets (PNNs) are specialized regions of the extracellular matrix, frequently found surrounding the soma and proximal dendrites of fast-spiking parvalbumin expressing (PV+) interneurons. Their appearance overlaps the maturation of neuronal circuits and the closure of critical periods in different regions of the brain, setting their connectivity and reducing abruptly their plasticity. In the presentation I will review recent evidence of the impact of PNNs presence on the input and output connectivity of PV+ neurons and their physiology. I will also talk about the influence of psychiatric disorders on PNNs and on the inhibitory circuits in which they are involved.
Results: For this I will revise recent results from our laboratory on animal models involving adverse experiences, particularly in early life. Additionally, I will present results about alterations in PNNs and inhibitory networks in postmortem tissue from patients with schizophrenia, bipolar disorder or major depression. The focus of the presentation will be the cerebral cortex, but I will also explore other brain regions, such as the thalamus, in which PNNs have a relevant presence.
Conclusion: Together, all the data available point to PNNs as promising targets to understand the physiology of inhibitory circuits involving fast-spiking cells, and to unveil the etiopathology of certain psychiatric disorders CNS.
Key words: Inhibitory neurons, parvalbumin, extracellular matrix, perineuronal nets

S44. The structure and distribution of the extracellular matrix affect the movement and migration of brain cells.Dr. Federico N. Soria[1,2], Mario Fernandez-Ballester[1], Irene Tomé-Velasco[1,3], Maria Ardaya[1], Ander Baraibar[1,3].
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Beyond neurons and glia, the Central Nervous System (CNS) holds a plastic scaffold known as the extracellular matrix, which in the brain consists mostly on long chains of the glycan polymer hyaluronan. The neural matrix serves a dual function of structural framework and signalling hub, communicating via both mechanical and diffusible cues that can elicit different cell responses. We have shown recently that microglia, the never-resting immune cells of the CNS, sense matrix disruption as a damage-associated molecular pattern (DAMP), while having also a role in matrix phagocytosis and turnover. However, it is unknown whether changes in the structural matrix affect microglial dynamics, i.e., motility, and migration. Here we report on the interaction between microglia and hyaluronan, using time-lapse imaging in ex vivo paradigms to characterise microglia dynamics in response to matrix modification. 2-photon time-lapse imaging in acute slices of Cx3Cr1+/eGFP mice showed that microglial motility, ramification and territory surveyed are reduced upon hyaluronan fragmentation, with no changes when other matrix components are degraded. Viral-mediated expression of Neurocan-GFP, a hyaluronan fluorescent reporter, showed that IB4-labelled microglia move and migrate differently through diverse matrix densities in organotypic slices. This observation was confirmed in 3D-cultures, using artificial matrix scaffolds of varying stiffness. These results imply a clear effect of the extracellular matrix upon microglia physiology, and shed light on the dual role of hyaluronan as scaffolding polymer and pro-inflammatory signal in the CNS.
Key words: extracellular matrix, glia, two-photon microscopy

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S45. Integrity of neural extracellular matrix is required for microglia-mediated synaptic remodeling. Dr. Carla Cangalaya[1], Weilun Sun[1,2], Stoyan Stoyanov[1], Ildiko Rita Dunay[3,4], Alexander Dityatev[1,4,5]

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Introduction/Objectives: Microglia continuously remodel synapses, which are embedded in the extracellular matrix (ECM). While the precise mechanisms governing this interplay remain elusive, this study aimed to understand the role of the neural ECM in microglia-mediated synaptic remodeling.
Materials and Methods: To investigate the influence of the neural ECM on synaptic remodeling by microglia, we disrupted ECM integrity by injection of chondroitinase ABC (ChABC) into the retrosplenial cortex of healthy adult Cx3cr1Cre/Tomato x GFPM Thy-1 transgenic mice.
Results: In vivo two-photon microscopy revealed that ChABC treatment significantly increased microglial branching complexity and phagocytic capacity for ECM components, while simultaneously decreasing basal spine elimination rates. Furthermore, attenuation of the ECM via ChABC treatment largely prevented microglial remodeling of synapses following photodamage-induced synaptic stress at single synaptic elements. These observations were accompanied by diminished stability and size of microglial contacts at damage sites, reduced deposition of calreticulin and complement proteins (C1q and C3) at synapses, and impaired microglial CR3 receptor expression.
Conclusions: Collectively, these findings provide novel insights into the critical role of the neural ECM in facilitating the deposition of complement proteins, thereby supporting microglial sculpting of synapses under physiological conditions.
Keywords: extracellular matrix; microglia; spine; synapse; complement protein; two-photon microscopy; C1q; C3

Symposium 12. ENVIRONMENTAL FACTORS IN THE PATHOPHYSIOLOGY OF CARDIOVASCULAR DISEASES.
Chair: Prof. Devendra K. Agrawal – *Western University of Health Sciences, USA*; Prof. Dragan M. Djuric – *University of Belgrade, Serbia.*

S46. Pathophysiological effects of the environmental factors and treatment strategies in cardiovascular diseases. Dr. Devendra K. Agrawal, Fihr Chaudhary, Zubair Ahmed.

Department of Translational Research, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, Pomona, California, USA

Introduction/Objetives: Despite many preventable risk factors, cardiovascular disease is the leading cause of death worldwide. Novel therapeutics have proven effective in controlling cardiovascular disease, yet risk factors for the disease remain a concern with poor outcomes evident across the globe. The continuous environmental factors, including air pollution, water pollution, climate change, industrialization, soil pollution, outdoor light pollution, lack of physical exercise, and environmental noise, are the major risk factors. In this presentation, underlying pathophysiological mechanisms due to environmental factors together with the public health policy, preventive measures, and treatment strategies to control cardiovascular disease will be presented.
Materials and methods: A literature search was conducted using PubMed and Google Scholar. Peer-reviewed full-length articles only in English language were selected based on several relevant keywords.
Results: Exposure to current levels of ambient particulate matter and stress due to other factors, both in the short and long term, is associated with an elevated risk of cardiovascular events. Increased coagulation/thrombosis, predisposition to arrhythmias, sudden narrowing of arteries, systemic inflammatory reactions, and the persistent promotion of atherosclerosis and resulting vascular damage are some of the underlying mechanisms. There is a complex interplay between genetic and environmental factors in the underlying pathogenesis, suggesting the role of genetic variants, hereditary factors, and environmental exposures. Many anti-inflammatory molecules, including aspirin, colchicine, lipid and glucose-lowering agents, and inhibitors of IL-1β, IL-6, TNF-α, PCSK-9, SGLT2, Activin A, xanthine oxidase, are potential treatment strategies.
Conclusion: Detrimental effects on cardiovascular health due to several environmental factors and genetics has become a global health problem. Both short and long-term actions are urgently needed to mitigate the current crisis. Further investigations for novel and effective anti-inflammatory agents in combination with cardioprotective molecules are warranted.
Key words: Anti-inflammatory agents, cardiovascular disease, environmental factors, genetics

S47. Homocysteine: a potential biomarker of environmental health risk and links to cardiovascular pathogenesis. Dr. Dragan M. Djuric – *University of Belgrade, Serbia.*

Homocysteine (Hcy) is a byproduct of the metabolism of the essential amino acid methionine. It's a sulfhydryl amino acid that cells naturally produce during the one-carbon metabolism process, which involves the transfer of methyl groupsa classic example of the interplay between genes and the environment. This metabolic pathway relies on various vitamins (B2, B6, B9/folate, B12), enzymes, and gasotransmitters. Hyperhomocysteinemia (HHcy), or high levels of Hcy in the blood, can result from physiological factors like aging, male sex, or increased muscle mass, genetic factors such as polymorphisms or defects in enzymes like CBS or MTHFR, unhealthy lifestyle choices (poor nutrition, drugs, pollutants, stress, physical inactivity), or certain clinical conditions (cardiovascular diseases, neurodegenerative diseases, kidney diseases, hypothyroidism, and hyperproliferative diseases). Elevated Hcy levels can lead to various detrimental effects, including alterations in DNA methylation, cellular toxicity (especially from thiolactone metabolites) causing cardiotoxicity and neurotoxicity, oxidative stress from reactive oxygen species (ROS) and reactive nitrogen species (RNS), oxidation of LDL cholesterol, inflammation, apoptosis, inhibition of certain enzymes, inhibition of cellular respiration, and changes in protein structure and function (protein S- and N-homocysteinylation). Ongoing research is focused on further elucidating the mechanisms underlying the relationship between homocysteine and cardiovascular pathogenesis. This includes investigating the potential role of epigenetic modifications, such as DNA methylation, in mediating the effects of homocysteine on cellular function and disease development. Understanding the intricate interplay between

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genetic, environmental, and lifestyle factors in modulating homocysteine levels is crucial for developing effective preventive and therapeutic interventions.
Key words: biomarker, environmental risk, homocysteine

S48. Novel approaches in reversing environmental stress initiated pathological processes to accelerate organ repair - the power of small molecules. Dr. Ildiko Bock-Marquette[1], Ferenc Gallyas Jr.[1], Szilard Rendeki[2], Gabor Faskerti[1], Balint Lippai[1]
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Introduction/Objetives: Social and environmental stress impose a significant burden upon our daily life often which accelerates through various inflammatory processes into severe pathological alterations. Although the human body has numerous repair mechanisms in response to these insults, the outcomes are too frequently insufficient. The mammalian heart is highly sensitive to stress-initiated processes and bears a limited capacity for self-renewal. Currently, many approaches are employed to restore its pathological alterations including application of progenitor cells and recently, reprogramming of locally available cells into functional cardiomyocytes with varying degrees of success.
Materials and methods: In our research we delve into the diverse aspects of how environmental stress triggers pathological processes at systemic, cellular and molecular levels. We primarily focus on the effects of physical and psychological stress upon the expression of various circulating molecules in the blood.
Results: We revealed secreted small molecules may serve as powerful tools not only to successfully track developing pathologies but also to aid reparatory processes. Thymosin beta-4, a 43 amino acid long secreted peptide proved especially significant in this aspect. **Conclusion:** Identifying small molecules is noteworthy not only for detecting the actual state of the human body but also for their potential use as "healers" for damaged organs. Utilizing these naturally secreted candidates may ultimately result in improvement of altered function thus potentially accelerating quality of life. We strongly believe our collective efforts inevitably result in improving organ function and regaining high quality lifespan for those weakened.
Key words: stress, cardiac regeneration, biomarkers, small secreted molecules, thymosin beta-4

S49. Isoprenaline model as stress induced myocardial injury. Dr. Zorislava Bajic[1], Tanja Sobot[1], Dragan M Djuric[2], Milos P Stojiljkovic[3], Ranko Skrbic[3].
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Introduction/Objectives: Isoprenaline, a beta-adrenergic agonist, is often used to induce experimental myocardial injury. It can cause cardiac disfunction and effects similar to those of emotional and physical stress in humans expressed as: increased heart rate, electrophysiological changes, and oxidative stress deterioration.
The aim of the study was to analyse functional, biochemical and morphological characteristics of isoprenaline-induced myocardial injury in rats.
Materials and methods: Male Wistar rats were divided into two groups: control and isoprenaline treated rats. Isoprenaline, given in two doses of 85 mg/kg with 24 hour interval, was used to induce myocardial injury. Cardiac function was analysed by ECG, blood was taken for biochemical analysis, and hearts were used for histological and immunohistological analysis.
Results: Isoprenaline induced an increase in specific biochemical parameters: troponine I (TnI), aspartate aminostransferase (AST), alanine aminostransferase (ALT), homocysteine and oxidative stress markers like plasma thiobarbituric acid reactive substance (TBARS), hydrogen peroxide (H_2O_2). At the same time the level of catalase (CAT) and reduced gluthation (GSH) were decreased. There were also changes in myocardial morphology with evident signs of inflammation, interstitial edema and bleeding. The right ventricular wall thickness and cardiomyocyte diameter were increased. There were evidence of isoprenaline induced apoptotic activity presented with TUNEL assay which showed an increase in DNA fragmentation, and with increase in proapoptotic markers BCL-2-associated X protein (BAX), cleaved caspase 3 (CC3), and nuclear factor kappa B (NF-κB), and the decrease in antiapoptotic marker B-cell lymphoma/leukemia-2 (BCL-2).
Conclusion: Isoprenaline model of myocardial injury can be used as an appropriate model to study the stress induced myocardial injury in humans.
Key words: isoprenaline, myocardial injury, oxidative stress, apoptosis

ORAL PRESENTATIONS

O1 Twenty-four weeks of moderate-intensity, but not vigorous-intensity, exercise training modulates the plasma lipidome in young adults: a secondary analysis from the ACTIBATE randomized controlled trial
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Introduction/Objetives: Exercise is a non-pharmacological intervention that may enhance cardiometabolic health, partially through the modulation of lipid homeostasis. However, how exercise modifies plasma lipid species in response to long-term exercise training has not been studied. Thus, we aimed to study

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the impact of a 24-week supervised exercise program at moderate (MOD-EX) and vigorous (VIG-EX) intensities on the plasma lipidome in young adults.
Materials and methods: This study was based on secondary analyses of the ACTIBATE single-center unblinded randomized controlled trial. Eligible participants were young, sedentary adults with no chronic diseases. Participants were assigned to either (i) a control group (no exercise, n=35), (ii) a MOD-EX group (n=32), or (iii) a VIG-EX group (n=34). 794 plasma lipids of 18 lipid classes were analyzed using targeted lipidomics. Adiposity and cardiorespiratory fitness (VO2max) were measured through standard methods. Responders in adiposity and VO2max were considered within-individual changes exceeding a Cohen d of 0.2.
Results: Changes in plasma lipidome were discriminant between groups (partial least squares-discriminant analysis performance=0.6). Concretely, MOD-EX increased glycerophosphoethanolamine (30% of phosphoethanolamines and 65% of lyso-phosphoethanolamines), phosphocholine (23%), phosphatidylinositol (10%), and triacylglycerol (12%) species compared to control group (all $P<0.05$). No differences were observed between VIG-EX and control group. VO2max responders to the exercise intervention showed higher changes in glycerophospholipids species than non-responders (all $P<0.05$). No differences were observed between responders and non-responders in adiposity.
Conclusions: 24 weeks of moderate-intensity concurrent training increased glycerophospholipids and triacylglycerol species. Glycerophospholipids might be involved in cardiorespiratory fitness improvements after the exercise intervention.
Key words: exercise training, lipidomics, body composition.

O2 Role of lingual CD36 in fat oro-sensory perception: tongue-brain axis

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Introduction/Objetives: Dietary lipids, in western diet (over 40%), contribute to the prevalence of obesity. Fat taste receptors, CD36 and GPR120, are expressed in taste bud cells (TBC) and play an important role in oro-sensory detection of long-chain fatty acid (LCFA). CD36 is crucial for the early detection of dietary fatty acids, whereas GPR120 controls postprandial regulation of fat ingestion. The gustatory message is sent via afferent VII and/or IX cranial nerves to the brain, particular to the nucleus tractus solitarius (NTS). The NTS further sends gustatory information to different regions of the brain which modulates eating behavior. However, the role of lingual CD36 in the orosensory perception of dietary fat remains to be clarified.
Materials and methods: We developed a cre-lox mouse model with a specific deletion of CD36 in TBC (TBC-CD36 -/-). We used two-bottle choice paradigm and licking test for fat preference. We evaluated c-fos expression in neurons by immunohistochemistry on brain slice.
Results: We observed, that TBC-CD36 -/- mice exhibited a significant downregulation of CD36 expression and an increase in GPR120 expression in circumvallate papillae. Likewise, LCFAs induced a weak increase in free intracellular Ca^{2+} concentration in TBCs from TBC-CD36 -/- mice as compared to TBC of control mice. Moreover, TBC-CD36 -/- mice exhibited a decrease in linoleic acid preference. This reduction in oral lipid sensitivity in TBC-CD36 -/- mice is likely due to reduced transmission of taste signal from the tongue to the brain. Indeed, we observed that the lingual application of LA in TBC-CD36 -/- mice weakly activates the NTS and ventral posteromedial nucleus of the thalamus (VPM) of brain areas compared to control mice as illustrated by decreasing of c-Fos expression in these regions. Finally, CD36 seems play an important role in TBC differentiation. We observed that expression of Shh and B-catenin, two markers of proliferation and differentiation of progenitor cells into TBC, was reduced in papillae of TBC-CD36 -/- mice.
Conclusion: These results demonstrate that lingual CD36 play a critical role in orosensory detection of dietary fat and impairment of CD36 expression in TBC alters fat-eating behavior. Lastly, the absence of CD36 in taste buds may induce an alteration in the differentiation of progenitor cells into mature TBC, which may have an impact on the nervous taste message.
Key words: Fat taste, taste bud cells, eating behavior, Brain

O3 Impact of Caloric Restriction on Beta Cell Function in Diet-Induced Type 2 Diabetic Mice

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Introduction/Objectives: Caloric restriction was effective in achieving remission of type 2 diabetes (T2D) in humans, but the underlying mechanisms at the beta cell level remain unclear. This study aims to elucidate the impact of caloric restriction on beta cell function in a mouse model of diet-induced obesity (DIO), which closely mimics human T2D.
Materials and methods: We developed a DIO mouse model using C57BL/6J mice fed a Western diet (WD) for 12 weeks, starting at 12 weeks of age. After this period, male mice exhibited T2D symptoms: obesity, insulin resistance, and glucose intolerance. We subjected the mice to a 7-day caloric restriction regimen (35% of normal caloric intake) to investigate the resultant changes in beta cell function and morphology.
Results: Mice displayed hyperinsulinemia, fasting hyperglycemia, and impaired glucose clearance, indicating early T2D with insufficient beta cell compensation. Caloric restriction completely reversed these symptoms, normalizing body mass, insulin sensitivity, fasting glycemia, glucose tolerance, and insulin levels. Functional multicellular confocal calcium imaging on pancreatic tissue slices revealed that DIO induced a left shift in glucose dependence in beta cells, contributing to hyperinsulinemia. Caloric restriction reversed this shift, reducing beta cell activity at given glucose concentrations to control levels.
Conclusion: Our findings suggest that in the early stages of T2D development, beta cells are hyperresponsive rather than dysfunctional. The glucose sensitivity shift is rapidly reversible with short-term caloric restriction. This study provides insights into the mechanisms by which caloric restriction impacts T2D and offers a robust animal model for further research on beta cell function and T2D reversibility.

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Key words: Caloric restriction, beta cell function, type 2 diabetes, diet-induced obesity, glucose sensitivity, insulin resistance, mouse model.

O4 Postnatal exposure to emerging endocrine disruptor chemical present a risk factor for gastrointestinal disorders in mice

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Introduction/Objetives: The gastrointestinal (GI) system is a complex physiological system dependent on genetic factors, gut-brain axis and exposome that refers to the totality of external and internal exposures in all life stage including chemical agents and biological agents. Growing evidence has shown that Endocrine-disrupting chemicals (EDCs), as an important part of the exposome may adversely affect GI system. In this context, limited informations are available on The gastrointestinal (GI) system is a complex physiological system dependent on genetic factors, gut-brain axis and exposome that refers to the totality of external and internal exposures in all life stage including chemical agents and biological agents. Growing evidence has shown that Endocrine-disrupting chemicals (EDCs), as an important part of the exposome may adversely affect GI system. In this context, limited informations are available on the effects of Bisphenol S, considered as an emergent EDC and as a food chain contaminant. It is frequently used as a plasticizer to manufacture various food-packaging materials. The aim of this study is to investigate the effect of Bisphenol S on apelin/APJ system as an important physiological factor in the gastrointestinal tract.
Materials and methods: Male mice were exposed to Bisphenol S (25, 50, 100 µg/kg, n=6 per group) via drinking water from PND 21 until 10 weeks post-lactation to simulate childhood exposure. Animals were treated with ethical guidelines. At the end of the treatment, fasted animals were sacrificed. After dissection, colons were rapidly removed and fixed with RNAlater and stored at -80°C. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was performed with LightCycler 480 using SYBR Green Real-Time PCR Master Mixes (ThermoFisher Scientific) and specific primers. The statistical significance of differences was analyzed by one way ANOVA followed by post hoc Bonferroni's. Results were considered statistically significant when $p<0.05$.
Results: Results show that Bisphenol S exposure does not affect inflammatory biomarkers genes but can alters transcript levels of APJ in colon at 50 µg/kg. Apelin/APJ system alteration can disrupt colonic transit that is an APJ receptor-depending mechanism.
Conclusion: Postnatal exposure to Bisphenol S at low doses present a risk factor for gastrointestinal disorders.
Key words: Bisphenol S, Endocrine-disrupting chemicals, Health hazards, Apelinergic system, Colonic transit

O5 Penile Endothelial Dysfunction and Impaired Redox Metabolism and Mitochondrial Bioenergetics in Diet-Induced Obesity: Compensatory Role of H_2O_2

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Introduction/Objectives: Vasculogenic erectile dysfunction is considered as a sentinel marker of cardiovascular disease and is associated with risk factors such as obesity. Mitochondria constantly produce reactive oxygen species (mtROS) through the ETC, that participate in endothelial signalling and control vascular tone (Widlansky & Gutterman, Antioxid. Redox Signal. 15, 1517, 2011). The aim of this study was to assess the impact of obesity on endothelial function and mitochondrial bioenergetics of penile arteries.
Materials and methods: Wistar rats were fed with a high-fat diet (HFD) or standard diet (STD), and penile vascular function was determined in microvascular myographs. To investigate the involvement of mtROS, the effect of the mitochondrial antioxidant mitoTempo on endothelium-dependent relaxations was tested. mtROS production was determined by mitoSOX (O2.-) and Amplex Red (H2O2) fluorimetry. Mitochondrial respiration of intact microarteries was assessed with an Agilent Seahorse XF Pro analyzer.
Results: NO-mediated endothelial relaxations to acetylcholine and to the mitoKATP channel activator BMS191095 were reduced in penile arteries from HFD. mtROS levels including H2O2 were significantly increased associated with upregulation of the endothelial Nox4 in HFD erectile tissue. MitoTempo inhibited endothelial relaxations in the controls and to a greater extent in HFD. Basal mitochondrial O2 consumption, as well as O2 consumption for ATP synthesis, proton leak, and non-mitochondrial respiration, were significantly reduced in HFD penile arteries.
Conclusion: Obesity impairs the relaxing function of mitoKATP channels in penile arteries, reduces mitochondrial bioenergetics, and promotes an adaptive mechanism in response to decreased NO-dependent endothelial relaxation through an increase in mitochondrial-derived vasodilator H2O2.
Keywords: mitochondria bioenergetics, penile endothelium, obesity
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O6 Alteration of placental and umbilical cord plasma oxidative stress in maternal obesity

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Introduction/Objetives: Maternal obesity has been found to impair oxidative status in the placenta and newborns, which can lead to adverse pregnancy outcomes and long-term adverse influences on metabolic pro-gramming in offspring. This study aimed to investigate the role of maternal obesity on maternal and umbilical cord plasma oxidative status and placental oxidative adaptation.
Materials and methods: Maternal obesity (n=14), defined as prepregnancy BMI ≥ 25 kg/m2, and maternal lean (n=14), defined as prepregnancy BMI < 25 kg/m2, were recruited into this study. Both groups were matched by gestational age at delivery. Maternal blood, cord blood and placental tissue were collected immediately after delivery for assessment of

oxidative stress markers (MDA) and antioxidant markers (catalase activity). Placental protein expression (SOD2, catalase, UCP2) was evaluated by western blot analysis.
Results: Catalase activity in maternal plasma significantly increased in the maternal obesity group ($p = 0.0264$) without a change in MDA levels. Umbilical cord plasma MDA and catalase activity significantly increased in maternal obesity compared with that in maternal lean ($p = 0.0007$). Placental protein expression analysis revealed decreased SOD2 (Mn-SOD) and catalase with increased mitochondrial antioxidant UCP2 expression.
Conclusion: Maternal nutrition plays an important role in maternal feto-placental oxidative balance. Maternal obesity is associated with fetal oxidative stress with the parallel increased placental antioxidants which may contribute to fetal metabolic programming. The results will provide scientific evidence to perform nutritional counselling to women in reproductive age to improve maternal-fetal health and reducing the impact of fetal programming in the future generations.
Key words: maternal obesity, placenta, oxidative stress, antioxidants

O7 Gut-brain axis modulation prevents diet-induced cognitive dysfunction, inflammation and oxidative stress in rat brain

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Introduction/Objetives: Western diets (WD) high in fat and sugar promote brain dysfunction, therefore the individuation of strategies capable of preventing via the gut-brain axis diet-induced impairment of brain physiology represents a mandatory challenge. The aims of the study were to investigate the impact of a high fat and fructose diet on hippocampus and frontal cortex of WD-fed rats and the efficacy of the probiotic *Limosilactobacillus reuteri* DSM 17938 (*L.reuteri*) in counteracting diet-induced dysfunction.
Materials and methods: Male Wistar rats were fed a western diet for 8 weeks, without (WD group) or with the administration of *L.reuteri (*WDR*)* and compared to control rats (C). Memory test on all rats, brain protein analysis by western blotting, activity of antioxidant enzymes, ELISA and mass spectrometry were performed on brain areas.
Results: *L. reuteri* prevented the WD-associated impairment in brain memory and consistently increased molecular markers of synaptic plasticity. The WD-induced condition of neuroinflammation and oxidative stress, namely enhanced oxidation to proteins and lipids, increased oxidants-producing enzymes as well as reduced nuclear Nrf2 and Nrf2-dependent antioxidant enzymes, was ameliorated by the probiotic administration. The WD induced a microbiota reshaping, but *L. reuteri* neither modulated this change, nor the plasma levels of short chain fatty acids, so its efficacy was attributable to the regulation of WD-induced metabolic endotoxemia and systemic inflammation, as decreased levels of LPS and plasma cytokines were found. A protective effect of the probiotic on gut absorption of fructose and its delivery to brain was evidenced as a mechanism underlying its activity and involved in brain homeostasis.
Conclusion: The findings evidence the efficacy of *L. reuteri* against WD-induced brain dysfunction and implicate the Nrf2 pathway as a potential mechanism for this effect.
Key words: fructose; oxidative stress; memory

O8 Title Diet-induced impairment of skeletal muscle and adipose tissue metabolic homeostasis and its prevention by probiotic administration

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Introduction/Objetives: Western dietary pattern is one of the main contributors to the increased risk of obesity and chronic diseases, through oxidative stress and inflammation in the most relevant metabolic organs, such as skeletal muscle. Indeed, the chronic exposure to high levels of dietary fatty acids can increase the amount of intramyocellular lipids in skeletal muscle, altering glucose homeostasis and contributing to reductions in muscle protein synthesis and mitochondrial oxidative capacity. Probiotics administration is a promising approach as preventive strategies to attenuate Western diet induced metabolic damage. Therefore, we verified the potential beneficial effect of *Limosillactobacillus Reuteri* DSM 17938 on the inflammatory state and oxidative balance in the skeletal muscle and adipose tissue in adult rats fed a western diet (8wks), focusing on the role of skeletal muscle mitochondria.
Materials and methods: Male Wistar rats of 90 days were divided in 3 groups, fed a control diet (C), a western diet alone (W) or in combination with the administration of 10^8 CFU of *L. reuteri* (W-R) for 8 weeks. Energy balance and body composition, glucose homeostasis and insulin sensitivity, and skeletal muscle and adipose tissue homeostasis were evaluated, with mitochondrial function in the skeletal muscle.
Results: We here demonstrate for the first time that *L. reuteri* DSM 17938 has a strong efficiency in protecting the skeletal muscle from mitochondrial dysfunction and oxidative stress, preventing the establishment of inflammation and insulin resistance. Interestingly, it exerts a beneficial effect on the body composition, favoring the deposition of protein mass and preventing adipose tissue hypertropy and inflammation, exhibiting a potential anti-obesity effect.
Conclusion: *L reuteri* DSM 17938 can be an effective probiotic in preventing the unhealthy consequences of the WD, opening to the formulation of new preparations as a therapeutic approach for nutrition-related diseases.
Key words: skeletal muscle, adipose tissue, diet, inflammation

O9 A specific matrisome composition drives a regeneration program in peripheral nerve repair

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Introduction/Objetives: Peripheral axonal neuropathies are a common reason of disability due to the progressive axonal impairment. Even if peripheral neurites are able to regenerate after damage, several patients exhibit progressive worsening without evidences of regeneration. The coordinated and balanced interaction between the Schwann cells, the re-growing axons, the surrounding extracellular matrix (ECM) and the blood vessels takes part in a series of events that eventually results in nerve repair. Most of the studies focused their attention on neurons and Schwann cells. However, evidence indicates that regeneration in the PNS mainly depends on the permissive environment in the endoneurium, suggesting a primary role of ECM in this context. Peripheral nerve regeneration is a great challenge in regenerative medicine that requires the local regulation and coordination of biological processes and molecular networks implemented by all the microenvironment's cellular components, including the ECM. The general alteration of the matrisome can affect the clinical outcome of nerve regeneration. Little is known about the ECM composition in human peripheral neuropathies and their potential role in promoting, or hampering, nerve repair.
Materials and methods: By means of shotgun proteomics, we investigated the matrisome composition of human sural nerve biopsies from patients with peripheral neuropathies exhibiting: i) acute axonal injury, ii) severe chronic neuropathy or iii) axonal regeneration.
Results: We described protein alterations that occur in these physiopathological contexts, revealing a specific signature for each condition. To mention a few, an apolipoprotein cluster (i.e. ApoD, ApoE, ApoA1) was uniquely overexpressed in nerves with acute axonal damage and different collagen isoforms appeared to be specifically up-regulated in nerves with regenerating axons or severe chronic damage.
Conclusion: Overall, this study provides a functional connection between the matrisome composition and peripheral nerve regenerative profile.
Key words: ECM, Apolipoproteins, peripheral nerve regeneration.

O10 Cognitive Impact of Central Auditory Processing on Listening Effort: Insights from a Simultaneous EEG and Pupillometry Study

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Introduction/Objetives: Although a limited number of studies examine the relationship between central auditory processing skills and listening effort, no study has investigated the relationship between different listening effort measurement methods and central hearing skills. The simultaneous implementation of listening effort assessment methods is very important in order to ensure that the participant is at the same motivation and neural resource level. This study aims to examine the relationship between simultaneous listening effort measurements and central auditory processing skills.
Materials and methods: Forty-nine individuals between the ages of 19 and 34 with normal hearing participated in the study. The study was carried out in two stages. In the first stage, simultaneous pupillometry, EEG, and single-task paradigm reaction time (RT) measurements were made during the challenging listening and repetition task with the impaired speech stimulus (noise-vocoded speech). Pupil dilation change and EEG alpha power change values used the measure listening effort. Two speech reception thresholds (SRT) (50% and 80%) for two vocoding conditions (16 and 6 channels) were collected, resulting in four conditions. In the second stage, at the same time of the day and the same level of fatigue as the first stage, the Dichotic Listening Test (DL), random gap detection (RGD), and frequency pattern test (FP) were applied. Stepwise linear regression analyses were conducted to examine the predictors of listening effort measurements, the central auditory processing tests that exhibited statistically significant correlations with all listening effort measurements across each challenging listening condition.
Results: A significant relationship was found between all central auditory processing tests and listening effort measurements. When stepwise regression analyses were examined, significant predictors of pupil dilation change were found to be RGD and FP, while predictors of EEG alpha power change were found to be DL and FP.
Conclusion: The study found that the pupil dilation change was more sensitive to temporal processing skills, and the EEG alpha power change was more sensitive to binaural fusion. This study is the first to investigate the sensitivity of different listening effort measurement methods to central auditory processing skills.
Key words: listening effort, central auditory proccesing, eeg alpha power, pupillometry

O11 Assessment of auditory and visual attention in young adults according to gender difference

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Introduction/Objetives: Some studies have shown that gender differences influence attention. In this study, we used multitasking to assess auditory and visual attention in relation to gender differences.
Materials and methods: The study included 86 participants (46 females, 40 males) aged 18-24 years. Participants were asked to perform a monkey business illusion test (visual attention test) with a song with pure sounds hidden in the background. On-ear headphones were worn by the participants and the song and video were played at the same time. While participants were watching the video with music and pure tone stimuli in both groups, requested to count the number of women wearing white t-shirts.
Results: As a result of the comparison made within the fully aroused group in terms of visual and attention according to gender, no statistically significant difference was found between male and female subjects in terms of recognizing the gorilla, the curtain color and the girl coming out of the stage, and recognizing the pure tone stimuli at 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz and 6000 Hz.
Conclusion: The effect of the gender factor both on auditory and visual attention was also investigated. As a result of these analyses, it was seen that there was no statistically significant difference between girls and boys on attention. Although the results in the literature vary, in many studies, gender difference is not found to be effective on attention as in this study.
Key words: attention, multitask, monkey business illusion test

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O12 Antioxidant effects of docosahexaenoic acid in an experimental model of multiple sclerosis

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Introduction/Objetives: Multiple Sclerosis (MS) is a chronic autoimmune, inflammatory, and neurodegenerative disease of the central nervous system of unknown origin, which causes oxidative stress in nervous and non-nervous organs. However, nutritional factors are known to affect the pathological mechanisms of MS, its development, and its course. Docosahexaenoic acid (DHA), provided by food, has emerged as an important player in the regulation of neuroinflammation by exerting a neuroprotective effect. This study aimed to evaluate the effect of DHA supplementation on clinical state and oxidative stress produced by experimental autoimmune encephalomyelitis (EAE), an animal model of MS.
Materials and methods: 25 Dark Agouti rats were used, which were divided into Control Group, Control+Vehicle Group, Control+DHA Group, EAE Group and EAE+DHA Group. DHA was administered for 51 days by intraperitoneal injection at a dose of 40 mg/kg, once a day, five days a week. The clinical status of the animals, glutathione redox cycle and biomarkers of oxidative stress in brain, spinal cord, heart, liver, kidney, and intestines were analyzed.
Results: DHA decreases the main biomarkers of oxidative stress and increases antioxidant defenses, both in nervous and in non-nervous tissue and organs. Furthermore, DHA injection plays a role in neuroprotection, being able to decrease the clinical score of EAE. This may be due to the activation of the antioxidant factor Nrf2 by DHA.
Conclusion: DHA supplementation could have a beneficial effect on MS, although it is necessary to determine the dose, route, and time of administration necessary to reduce oxidative stress and neurodegeneration.
Key words: Experimental autoimmune encephalomyelitis, Omega-3, Oxidative Stress.

O13 Human neural stem cells communicate with each other through tunneling nanotubes, exchanging organelles and self-renewal signals

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Neural stem cells (NSCs) are self-renewing, multipotent cells capable of differentiating into neurons, astrocytes, and oligodendrocytes. Tunneling nanotubes (TNTs) are long plasma membrane bridges that physically connect distant cells, enabling the intercellular transfer of organelles and signals. Polypyrimidine tract-binding protein-1 (PTBP1) serves as a master regulator of neural stem cell differentiation by regulating alternative splicing and mRNA translation.
Whether human neural stem cells (hNSCs) communicate through TNTs and whether TNTs transfer organelles and stemness/differentiation signals remains unknown.
Here, using Tau-STED super-resolution confocal microscopy, live-cell time-lapse fluorescence microscopy, and direct or non-contact homotypic co-cultures between hNSCs, we demonstrated that hNSCs generate nestin-positive TNTs in both 3D neurospheres and 2D cultures, through which they exchange functional mitochondria.
To analyze the potential role of TNTs in the transfer of stemness/differentiation signals, we focused our attention on PTBP1. RNAi experiments revealed that PTBP1 knockdown significantly reduced the number and size of hNSC neurospheres, inhibited hNSC growth, and upregulated β3-tubulin, showing for the first time the pivotal role of PTBP1 in hNSC self-renewal and neuronal differentiation. Analyzing PTBP1 subcellular localization, we found that PTBP1 localizes in the nucleus, as expected, but also within the cytoplasm and inside F-actin and nestin-positive TNTs connecting hNSCs.
Our data describe the presence of a functional TNT network connecting hNSCs, through which hNSCs exchange mitochondria and probably stemness/differentiation signals. These findings contribute to understanding how stem cells control their self-renewal and differentiation.
Key words: Human neural stem cells, Tunneling nanotubes, Stemness and differentiation

O14 Altered basal phosphorylation levels of synaptic enzymes and glutamate ionotropic receptors across life: influence on hippocampal LTP.

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Introduction/Objetives: Hippocampal long-term potentiation (LTP) endures developmental adaptations during adolescence and ageing. These involve both postweaning maturation and late-life adaptations of GABAergic circuits and glutamatergic synapse composition[1], including AMPA and NMDA receptor composition. Since CA1 TBS-induced LTP relies on the Ca^{2+}-dependent auto-phosphorylation and activation of CaMKII[2] and the phosphorylation of AMPA receptor GluA1 subunits altering channel conductance and recruitment to the active zone, we investigated the phosphorylation levels of CaMKII, AMPA GluA1 subunits and NMDA GluN2B subunits across life in rats and its correlation with TBS-induced hippocampal CA1 LTP.
Materials and methods: The protein and phosphorylation levels of CaMKII, AMPA GluA1 and GluA2 and NMDA GluN2B subunits were detected by western-blot in hippocampal synaptosomes[1] from rats aged 3-weeks to 21 months.
Results: CaMKII phosphorylation at Thr177 gradually increased from weaning to adulthood, (at 3 months old, n=5) but decreased by 21,3% with afrom 4 to 21 months old (n=3). The adult GluA1 phosphorylation at Ser831 was nearly half the observed at weaning, decreased mildly with age but dropped markedly at 21 months (by 81.3%, n=3). AMPA GluA1 subunits and GluA1/GluA2 ratio nearly doubled during adolescence (n=5) but both GluA1 levels and GluA1/GluA2 ratio decreased mildly with ageing (n=4). GluN2B phosphorylation decreased mildly while GluN2B levels were markedly decreased (by 72,3%) from weaning to adulthood (3

months, n=5), decreased moderately throughout middle age and dropped strongly at 21 months (n=4).
Conclusion: CaMKII activity, promoting GluA1 and GluN2B phosphorylation, is larger at weaning and decreases progressively throughout life causing a gradual shift in LTP-inducing mechanisms that has remained overlooked. Decreased LTP in early adolescence might be caused by saturation of the synaptic phosphorylation pathways allowing enhancement of synaptic strength and suggest an hyperexcitable state, as occurs in epilepsy[3].
1. Rodrigues et al. (2021) Eur J Neurosci 54:5272; **2.** Appleby et al. (2011) J Neurochem 116:530; **3.** Cunha-Reis et al (2021) Pharmacol Res 164: 105356.
Key words: LTP; CaMKII, AMPA, NMDA; aging; adolescence.

O15 Expression of guanylate cyclase C and uroguanylin in the brain is regulated by feeding

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Introduction/Objetives: The expression of guanylate cyclase C (GC-C) in the brain is known for several decades. However, the presence GC-C agonists, like uroguanylin (UGN), has been disputed.
Materials and methods: In this study UGN expression was confirmed at mRNA (RT PCR, qPCR, RNAScope) and protein (Western blot, ELISA, immunohistochemistry) level in mouse brain. We also determine UGN and GC-C in human prefrontal and cerebellar cortex, arcuate nucleus of hypothalamus and substantia nigra in brain samples of human 21 male brains.
Results: GC-C was found in all tested brain areas and it was confirmed in neurons of the third cortical layer of Brodmann area (BA)9. On the other hand, UGN is expressed in all cortical layers in several interneuron subpopulations. Both UGN and GC-C expression in mouse and human brain was regulated by feeding. GC-C expression at protein level in male BA10 and BA11 was in negative correlation to the volume of stomach content during autopsy while UGN expression was downregulated after a meal in BA9 and 10 with no change in the BA11 and 32. Two hours after a meal UGN mRNA in mouse cerebral cortex was in positive corelation to glucose concentration in cerebrospinal fluid (CSF). In mouse hypothalamus, UGN mRNA was in negative correlation to glucose concertation in CSF.
Conclusion: These results confirm, without any doubt, presence of UGN in the mouse and human brain. UGN and GC-C expression is regulated by feeding suggesting possible involvement of UGN signalling pathway in feeding regulation in laboratory animals and cognitive regulation of feeding in humans.
This study was financed by the ERDF (KK.01.1.1.01.0007) and by the CSF (IP-2018-01- 7416).
Key words: human prefrontal cortex, hypothalamus, feeding behaviour

O16 Calcineurin B1 deletion in astrocytes rescues memory loss and histopathology in Alzheimer's disease mouse models

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Introduction/Objectives: Increasing Alzheimer's disease (AD) incidence and continuous failures of clinical trials call for exploring innovative therapeutic targets. AD has a complex etiology in which astrocytes, principal homeostatic cells of the central nervous system, are critical in pathogenesis. Indeed, at the early stage of AD reactive gliosis and neuroinflammation are reported and associated with the activation of calcineurin (CaN) in astrocytes. Interfering with the CaN pathway in astrocytes represents a promising cell target. Here, we aimed to investigate if deletion of the regulatory CaNB1 subunit of CaN in astrocytes could mitigate AD-related memory deficits, neuropathology, and neuroinflammation.
Materials and methods: We have generated two, acute and chronic, AD mouse models with astrocytic CaNB1 ablation (ACN-KO).
In the former, we evaluated the ability of β-amyloid oligomers (AβOs) to impair memory and activate glial cells once injected into the cerebral ventricle of conditional ACN-KO mice. Next, we generated a tamoxifen-inducible astrocyte-specific CaNB1 knock-out in 3xTg-AD mice (indACNKO-AD). CaNB1 was deleted, by tamoxifen injection, in 11.7-month-old 3xTg-AD mice for 4.4 months. Spatial memory was evaluated using the Barnes maze; β-amyloid plaque burden, neurofibrillary tangle deposition, reactive gliosis, and neuroinflammation were also assessed.
Results: The acute model showed that ICV injected AβOs in 2-month-old wild-type mice impaired recognition memory and fostered
a pro-inflammatory microglia phenotype, whereas, in ACN-KO mice, AβOs were inactive. In indACNKO-AD mice, 4.4 months after CaNB1 depletion, we found the preservation of spatial memory and cognitive flexibility, abolishment of amyloidosis, and reduction of neurofibrillary tangles, gliosis, and neuroinflammation.
Conclusion: Our results suggest that ACN is crucial for developing cognitive impairment, AD neuropathology, and neuroinflammation. Astrocyte-specific CaNB1 deletion is beneficial for both the abolishment of AβO-mediated detrimental effects and the treatment of ongoing AD-related pathology, hence representing an intriguing target for AD therapy.
Key words: Astrocytes, calcineurin, Alzheimer's diseas

O17 Calcium physiology and pathophysiology in the heart of zebrafish larvae with fluorescent biosensors

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Introduction/Objetives: Zebrafish is a useful vertebrate model to study heart physiology and disease. Despite anatomical differences with the human heart, their electrophysiology and heart rate are very similar. We imaged calcium to study the function of T-type calcium channels

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(TTCCs), the small G-protein rrad, and phospholamban mutation R9C in live zebrafish larvae.
Materials and methods: The zebrafish line expressing ratiometric fluorescent calcium biosensors allowed measurements of contraction and calcium levels in beating hearts in larvae (doi:10.7150/thno.64734). TTCC and rrad gene knockout larvae were generated with CRISPR-Cas9. Transgenic lines overexpressing rrad and phospholamban b (wild-type and Arg9Cys mutation) were also raised.
Results: Larvae incubated with a TTCC blocker and *cacna1g* gene knockout larvae showed bradycardia, atrioventricular blocks and slow conduction, but no change in contractility, indicating that TTCCs are involved in automaticity of the pacemaker and atrioventricular conduction in zebrafish larvae, as in mammals.
The Ras-like small G-protein Rad mediates the regulation of cardiac Cav1/Cav2 channels by adrenergic signals in mammals. Rrad+/- zebrafish larvae displayed increased heart rate and calcium levels, whereas larvae overexpressing rrad showed reduced calcium levels, contractility, and cardiac output. Thus, rrad mediates calcium handling and inotropism by beta-adrenergic agonists in zebrafish heart.
Phospholamban inhibits the ATPase SERCA2a. Its point mutation R9C causes a dilated cardiomyopathy in humans. Zebrafish lines overexpressing wt- and R9C-phospholamban were produced. R9C-phospholamban increased the amplitude of the calcium transients, the inotropy and lusitropy, and blunted the beta-adrenergic response in 3-day larvae, but 9-day larvae exhibited ventricular dilation, systolic dysfunction and negative lusitropy, hallmarks of a dilated cardiomyopathy.
Conclusion: Zebrafish expressing gene encoded fluorescent calcium biosensors are excellent models to study cardiac physiology and pathophysiology.
Key words: calcium, zebrafish, heart

O18 The effect of acute hyperglycemia on microvascular reactivity challenged by iontophoresis of acetylcholine

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Introduction/Objetives: The effect of acutely elevated plasma glucose levels on microvascular reactivity in healthy individuals remain controversial. We investigated the effects of oral glucose loading (OGL) on microvascular endothelial function in healthy subjects using laser Doppler flowmetry (LDF) coupled with acetylcholine (Ach) iontophoresis as a perfusion quantification technique, and wavelet analysis (WA) as an interpretative tool.
Materials and methods: Ten healthy volunteers participated in a test protocol in which they drank a standard glucose solution (75 g dissolved in 250 ml water), and in a control protocol in which they drank the same amount of water.LDF recordings of Ach iontophoresis response in each protocol were acquired before and 45 minutes after each intervention. Acquired signals were decomposed into their frequency spectra using WA. The relative power (RP = median power of each frequency interval / median power of the total spectrum) was calculated to compare the contributions of different physiological mechanisms to the regulation of microcirculatory responses.
Results: WA detected periodic oscillations with frequencies corresponding to cardiac, respiratory, myogenic, neurogenic, endothelial NO-dependent, and endothelial NO-independent influences. OGL affected microcirculation differently than water, with significant differences in respiratory (0.43 pre-load; 0.68 post-load) (p=0.02; paired t-test test), myogenic (0.77 pre-load; 1.58 post-load) (p=0.02), endothelial NO-dependent (1.41 pre-load; 1.06 post-load) (p=0.05), and endothelial NO-independent (4.00 pre-load; 2.40 post-load) (p=0.02) components observed between the pre-load and post-load OGL periods.
Conclusion: OGL induces microvascular impairment, likely involving, but not limited to, modulation of low-frequency intervals related to vascular endothelial function.
Key words: endothelium, acute hyperglycemia, microcirculation

O19 Sex differences in aging-related miR-149-5p modulation of the angiogenesis pathway in experimental acute myocardial infarction

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Introduction/Objetives: Aging is an important risk factor for the development of acute myocardial infarction (AMI) which differentially affects men and women. miRNAs have been proposed as physiological regulators and in previous studies we observed a dysregulation of miR-149-5p in AMI. Our aim is to study the impact of sex and aging in the expression of miR-149-5p and its target genes in a Senescence-Accelerated Mouse (SAM) undergoing AMI surgery.
Materials and methods: AMI was surgically induced by coronary artery ligation in six-months-old male and female SAM-Prone (SAMP8; n=16) and its control, SAM-Resistant mice (SAMR1; n=16; Ethics 2020/VSC/PEA/0128). Sham-operated group was used as control of surgery. Animals were euthanized 4 hours after surgery and serum and heart were collected. RNA was isolated and levels of miR-149-5p and gene expression was determined by qRT-PCR. *In silico* analysis of miR-149-5p target genes was performed.
Results: miR-149-5p is higher expressed in both serum and heart tissue in male senescent mice. After 4h AMI, circulating miR-149-5p only increased in SAMP8 females ($p<0.05$), and heart expression levels decreased in SAMR1 and SAMP8 males ($p<0.001$). *In silico* analysis, associated miR-149-5p to angiogenesis pathway being FZD5 and PPAP2B predicted target genes. FZD5 and PPAP2B gene expression showed similar behaviour and increased after AMI ($p<0.01$) in heart tissue from all the mice groups except for female SAMP8.
Conclusion: In experimental AMI, aging is associated to angiogenesis pathway through the modulation of FZD5 and PPAP2B genes by miR-149-5p. There is also a sex-specific regulatory mechanism by miR-149-5p confirmed by the observed differences in serum and heart tissue from aged females.
Acknowledgements: Funded by the Spanish Ministry of Science and Innovation (ISCIII) PI22/1083 co-financed by the European Regional Development Fund (ERDF), and by the Generalitat Valenciana (CIAICO 2021/211; CIGE/2021/158). BDB is a predoctoral researcher (CIACIF/2022/331) from the Generalitat Valenciana.
Key words: Acute myocardial infarction, aging, miR-149-5p, sex differences

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O20 Alanine mutation of the targeting subunit of the myosin-light-chain-phosphatase, MYPT1 prevents development of hypercontractile phenotype of murine interlobar arteries

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Introduction/Objetives: Vascular tone of pre-glomerular arteries is the main determinant of glomerular filtration and plays a pivotal role in maintenance of blood pressure regulation. In the present study we explored the regulation of vascular tone of murine interlobar arteries (IAs) by phosphorylation of the targeting subunit of the myosin-light-chain-phosphatase, MYPT1.
Materials and methods: We utilized mouse model in which MYPT1 was targeted at Threonine 696 by a point mutation and replaced by non phosphorylatable alanine. Expression of MYPT1-T696 was determined by western bolt. Isometric tone of IAs from wild types (WT) and heterozygote animals carrying the mutation at MYPT1-T696 (MYPT1-T696A/+) were determined by wire myography.
Results: In IAs from MYPT1-T696A/+ mice alanine point mutation reduced expression of MYPT1-T696 by~50%. It didn't affected reactivity to alpha1-agonist, phenylephrine or endothelial responsiveness to acetylcholine of young IAs. In line with this the responsiveness of young IAs to direct activation of soluble guanylat cyclase by cinaciguat or RhoA-kinase inhibition by Y27632 was not affected in IAs from MYPT1-T696A/+ mice. In IAs from WTs, aging augmented phenylephrine responsiveness and attenuated those to cinaciguat and Y27632. By contrast, aged IAs from MYPT1-T696A/+ animals showed similar responsiveness to Y27632 as young IAs from WT.
Conclusion: Present study evidences that in young age the myosin phosphorylation plays a minor role for IAs-tone regulation. Our study supports the view that MYPT1-T696 alanine mutation prevents development of hyperreactive phenotype of IAs in aging and introduces a novel therapeutic concept for targeting of pathological pre-glomerular tone.
Key words: Pre-glomerular tone, MYPT1-T696, alanine point mutation

O21 Exercise-induced alterations of the murine heart during doxorubicin treatment of breast cáncer

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Introduction/Objetives: Exercise can attenuate some cardiotoxic effects of doxorubicin (DOX) in tumor-free animals. However, the exercise-effects on cardiac function and glucose-metabolism in DOX-treated breast cancer models are still unclear. The study aimed to investigate the exercise-induced structural, functional, and metabolic alterations of the heart in a murine breast cancer model during DOX-treatment.
Materials and methods: FVB/N female mice were subcutaneously inoculated with mammary tumor -derived I3TC-cells (day 0). Subset of these mice received DOX-chemotherapy (intraperitoneal 5 mg/kg/week, at days 8, 15, 22, 29 from inoculation) with half given access to voluntary running-wheel exercise. Similarly, tumor-bearing mice without DOX-treatment were assigned to unexercised and exercised. Mice underwent [18F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography and echocardiography at 20-day and 34-day from inoculation, and were euthanatized at day 35-37. DOX-treated mice were also compared with tumor-free unexercised and exercised controls. The cardiac effects were further investigated using histology and enzymatic assays.
Results: DOX increased left ventricular (LV) FDG-uptake and decreased LV-weight and ejection fraction. In DOX-treated mice, exercise lowered LV FDG-uptake, increased LV-weight and ejection fraction, and lowered LV lactate dehydrogenase activity. Exercised DOX-treated mice did not differ significantly from tumor-bearing group without DOX-treatment in LV FDG-uptake or from the tumor-free exercise group in LV-weight or ejection fraction whereas unexercised DOX-treated group did. Exercise also increased LV citrate synthase activity in all tumor-bearing animals.
Conclusion: In a murine breast cancer model, voluntary, spontaneous exercise training could blunt DOX-induced cardiotoxic effects such as DOX-mediated increase in cardiac FDG-uptake and decreased ejection fraction and LV-atrophy.
Key words: cardiac physiology, glucose-metabolism, cardiotoxicity.

O22 Role of lipoma-preferred partner in the phenotype control of vascular smooth muscle cells

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Introduction/Objetives: Hypertension continues to be one of the main causes of death worldwide contributing to ischaemic heart disease, stroke, and renal pathologies. Chronically elevated blood pressure increases arterial wall stress, and lipoma preferred partner (LPP), a Lin11–Isl1–Mec3 (LIM)-domain protein, is involved in sensing and/or transducing this mechanical cue. LPP is primarily expressed by vascular smooth muscle cells (VSMCs) and seems to maintain the quiescent contractile phenotype of these cells in the face of hypertension, whereby its exact mechanism of action remains indistinct.
Methods and results: In 2D and 3D models, primary aortic LPP-deficient (LPP-KO) VSMCs display higher rates of proliferation and motility but lower contractility compared to control cells. When exposed to cyclic stretch, LPP-KO VSMCs seem to adopt a proinflammatory expression profile. Own analyses of publicly available single-cell RNA sequencing data reveal that expression of LPP in patients with aortic aneurysms is significantly lower as compared to control subjects. Moreover, expression of LPP is significantly lower in human VSMCs with an activated synthetic phenotype as compared to cells with a quiescent contractile phenotype. When made hypertensive, LPP-deficient mice present with strong vascular pathologies, including aortic dissections as well as ruptures, that result in a high premature mortality

amongst male but not female animals. Moreover, functional analyses of isolated perfused third
order mesenteric artery segments of these mice show a significantly reduced myogenic response.
Conclusion: Overall, our data indicate an important role of LPP in VSMC phenotype control as well as in the adaptation of the vascular wall to increased wall stress.
Key words: LPP, hypertension, mechanotransduction, VSMCs, phenotype switch

O23 Effect of moderate hypobaric hypoxia on both heart and skeletal muscle during the first 24 hours of exposure.

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Abstract: Introduction/Objectives: Most of the data on mitochondrial adaptations, such as assemblies of mitochondrial transport chain complexes, following exposure to hypoxia come from in vitro experiments and from rodents subjected to hypobaric chambers, limiting conclusions to in vivo and natural exposure to environmental hypobaric hypoxia. Therefore, the purpose of the present study was to assess whether exposure to moderate hypoxia increases the assembly of mitochondrial supercomplexes in both skeletal and cardiac muscle.
Materials and methods: The normoxia situation was performed at the Biomedical Research Centre of University of Granada, at 630 meters above sea level. The hypoxia situation was performed at the High-Performance Altitude Training Centre of Sierra Nevada (CARD) located in Granada 2320 meters above sea level. Six rats were sacrificed in normoxia as control group. Eighteen male Wistar rats were exposed to moderate hypoxia and further divided into three groups differing in the length of hypoxia exposure: 0 hours group (n=8), 6 hours group (n=8) and 24 hours group (n=8). The rats were euthanized by overdose of anesthesia and the right gastrocnemius muscles were extracted. The evaluation of supercomplex assembly in gastrocnemius was assessed by blue native gel electrophoresis on crude mitochondrial fraction.
Results and Conclusion: We found a significant increase in mitochondrial supercomplex assembly in the heart as soon as the animals reached 2320m above sea level and their levels are maintained 24 h post-exposure, but not in skeletal muscle. In conclusion, mitochondrial supercomplexes increase in the heart, whereas in skeletal muscle, the mitochondrial pro-fission response is triggered.
Keywords: moderate hypobaric hypoxia, skeletal muscle, supercomplexes

O24 Lauric acid enhances endothelium-independent relaxation in the corpus cavernosum of streptozotocin-induced diabetic male Wistar rats

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Introduction/Objectives: Diabetes mellitus is a risk factor for erectile dysfunction. Erection involves the relaxation of the corpus cavernosum. Possible therapeutic properties of lauric acid have been reported. Hence, this study sought to investigate the relaxant effects of lauric acid on the corpus cavernosum of diabetic male Wistar rats.
Materials and Methods: Male Wistar rats were divided into five groups: control, diabetic untreated, and three diabetic groups treated with different doses of lauric acid (90 mg/kg, 180 mg/kg, and 360 mg/kg). Diabetes was induced with streptozotocin, and lauric acid was administered daily. After four weeks of treatment, the rats were euthanized, and their corpus cavernosum tissues were extracted. Following phenylephrine or potassium chloride (KCl)-induced contraction, the relaxation responses of the tissues to acetylcholine (endothelium-dependent) and sodium nitroprusside (SNP) (endothelium-independent) were then assessed.
Results: Relaxation of phenylephrine-contracted tissues to acetylcholine was significantly lower in diabetic untreated and treated groups compared to the control. Relaxation of KCl-contracted tissues to acetylcholine was significantly lower in diabetic untreated and treated groups with higher doses of lauric acid compared to the control. Relaxation of phenylephrine-contracted tissues to SNP was significantly lower in diabetic untreated and treated groups with the highest dose of lauric acid compared to the control. Relaxation of KCl-contracted tissues to SNP was significantly lower in diabetic untreated groups only, compared to the control.
Conclusion: Lauric acid improved the relaxation of corpus cavernosum in diabetic rats by endothelium-independent mechanisms; it enhanced the nitric oxide-mediated relaxing effect of sodium nitroprusside and possibly inhibited KCl-induced contraction.
Key words: Diabetes, Smooth muscles, Contractility

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O25 Fresh or fatigued assessment of VO2max? Preliminary observations on the durability of competitive, national-level male triathletes

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Introduction/Objectives: Maximal aerobic capacity (VO2max) after a *fresh* cardiopulmonary exercise test (CPET) is considered the gold-standard for assessing cardiorespiratory performance. However, in racing conditions, athletes are generally required to operate in an increasingly *fatigued* state. We hypothesize that *fresh* VO2max may be different from *fatigued* VO2max, with the former not fully capable to depict performance drops due to fatigue.

Materials and methods: Ten competitive, national-level male triathletes performed *fresh* cycloergometry CPET to assess both VO2max and the second ventilatory threshold (Vt2). Within 5-7 days they underwent a 40-minute fatiguing protocol by cycling steadily at Vt2 intensity, followed by a final ramp to reach their maximal performance. VO2, VE, VCO2, and HR were assessed during both tests.

Results: Fresh vs. fatigued CPET analysis showed a significant decline in maximal watts (-14%, $p<0.01$), VCO2 (-10%, $p<0.01$), and VE (-8%, $p<0.01$) during the fatigued test whereas VO2max and HR proved unchanged ($p=0.22$ and $p=0.27$, respectively). Correlation analysis showed a significant correlation between drops in wattages and those in VE ($r_s=0.93$, $p<0.01$) and HR ($r_s=0.78$, $p=0.04$).

Conclusion: VO2max was consistent from fresh to fatigued tests while only VCO2 and VE decreases allowed capturing the performance drops induced by the fatiguing protocol. Interestingly, athletes displaying comparable VO2max values exhibited different performance drops, i.e., different durability features. These preliminary data will need validation over larger samples and competition levels but seem to suggest that VO2max may be not as adequate as VCO2 and VE in describing the fatigued athlete performance.

Key words: VO2max, durability, anaerobic threshold

O26 Maximal myocardial blood flow and minimal vascular resistance in untrained and endurance-trained men

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Introduction/Objetives: Heart critically depends on continuous supply of oxygen, provided by myocardial blood flow (MBF), but MBF during maximal exercise has not been investigated in humans.

Materials and methods: In the present study we measured MBF at rest and during similar absolute (150 W) and relative (70% from VO2max) workload cycling exercise in seven healthy untrained men (UT) and seven healthy endurance-trained men (ET) by positron emission tomography (PET) and 15O-H2O-tracer. We determined their maximal MBF by extrapolation using their MBF, heart rate, and blood pressure data during PET measurements and maximal heart rate measured separately during maximal aerobic fitness test. We also measured their MBF during intravenous infusion of standard high dose adenosine known to cause maximal vasodilation in the myocardial vasculature.

Results: Maximal predicted MBF was 4.96±1.47 ml/g/min in UT and 4.45±1.48 ml/g/min in ET, which was 6.5 fold and 6.4 fold higher from their respective resting values. Adenosine-induced MBF was 3.88±1.36 ml/g/min in UT and 2.26±0.87 ml/g/min ET, being significantly lower than predicted maximal MBF during exercise in both groups. In addition, the group difference trended significant ($p=0.051$), being lower in ET. Myocardial vascular resistance (MVR) decreased significantly from rest to both adenosine- and exercise-induced vasodilation and it did not differ between the latter two conditions. However, MVR was significantly higher in ET compared to UT in both conditions.

Conclusion: Maximal MBF may be higher than previously considered, and higher than adenosine-induced MBF. Higher MVR in endurance-trained men suggests that they still have reserve for further vasodilation even during maximal exercise.

Key words: myocardial blood flow, exercise, training

O27 Accumulation of oral and inhaled nano and microplastics in various organs of male and female mice disrupts memory and leukocyte trafficking

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Introduction/Objetives: Plastics are increasingly produced and very difficult to degrade in nature. Although studies have shown that nanoplastics (<100nm) and microplastics (<5mm) cause immune system disorders, no study has evaluated the effects of nano/ microplastics on leukocyte trafficking. Therefore, this research aims to study the effects of nano/microplastics on leukocyte trafficking in male and female mice.

Materials and methods: 16 (female/male) C57Bl/6 mice were used. Experimental groups: (1) female/male mice not exposed to plastics; (2) female/male mice exposed to fluorescently labelled nanoplastics (40nm, 50µg/day/mouse) via inhalation and fluorescently labelled microplastics (30µm, 10µg/day/mouse) orally for 28 days. Behaviour tests were done two days before sacrification, and on the day of sacrification intravital microscopy (IVM) was performed. Tissues were collected and examined for plastic accumulation under fluorescent light. One-way ANOVA was used for sex-independent analyses, and two-way ANOVA was used for sex-dependent analyses.

Results: There were decreased short-term and spatial memory of mice exposed to plastics ($p<0.05$). Plastic accumulation observed in the brain, lungs and gastrointestinal tract. In IVM, a significant increase of leukocyte stationary ($p<0.05$) and adhesion ($p<0.05$) was observed. MDA levels were decreased in the liver, brain, and large intestine ($p<0.05$) while increased in the lungs ($p<0.05$). While GSH levels in the brain decreased ($p<0.05$), MPO levels tended to increase ($p=0.0938$).

Conclusion: Mice exposed to nano/microplastics showed impairments in short-term and spatial memory. Plastic accumulation was detected in many organs and plastic exposure was shown for the first time to cause disruptions in leukocyte behaviour. No sex-related differences were observed in these results.

Key words: Microplastic, nanoplastic, leukocyte trafficking

O28 Evolutionary-conserved temperature dependency leads to loss of protein O-GlcNAc in mammalian hypothermia

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Protein O-linked N-acetylglucosamine (O-GlcNAc) is a ubiquitous non-canonical glycosylation of intracellular proteins linked to numerous cellular processes. Only two enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), perform the addition and removal of this modification, respectively. The assumed primary mode of regulation of O-GlcNAcylation is the availability of its substrate uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), the end-product of the hexosamine biosynthetic pathway (HBP). We found a body-wide loss of O-GlcNAcylated proteins in *Bcs1l*$^{p.S78G}$ mice, modeling mitochondrial complex III deficiency. We suspected UDP-GlcNAc depletion due to HBP insufficiency as the cause. Indeed, all HBP precursors were decreased in the mutant liver. Unexpectedly, UDP-GlcNAc was only minimally decreased in the liver and not at all in some tissues showing drastic loss of O-GlcNAc. Neither N-acetylglucosamine supplementation nor overexpression of the HBP rate-limiting enzyme (GFAT1) restored the O-GlcNAc levels. Seeking an alternative mechanism, we learned that O-GlcNAc status has been shown to correlate with ambient temperature in some ectotherms (mainly in the fruit fly). The *Bcs1l*$^{p.S78G}$ mice became increasingly hypothermic during the disease progression, coinciding with declining O-GlcNAc. Transgenic expression of alternative oxidase (AOX), which can increase heat production by bypassing mitochondrial complexes III and IV, attenuated the hypothermia and the loss of O-GlcNAc. In adult fruit flies, zebrafish muscle, and several mammalian cell lines exposed to various temperatures, O-GlcNAc level showed a strong positive temperature dependency. Our results imply O-GlcNAc as an evolutionarily ancient intracellular thermometer.

Key words: Protein O-linked N-acetylglucosaminylation (O-GlcNAcylation), The hexosamine biosynthetic pathway, mitochondrial diseases

POSTER PRESENTATIONS

Cardiovascular Physiology

P1 Acute hypoxic vasodilation in systemic vascular smooth muscle depends on mitochondrial oxygen sensing and signaling.

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Introduction/Objectives: Oxygen (O_2) tension is a major regulator of blood flow in the systemic and pulmonary circulations. Voltage- gated L-type Ca^{2+} channels in vascular smooth muscle are O_2-sensitive and thus may critically contribute to acute arterial hypoxic vasodilation. However how variations of O_2 tension are detected and the mechanisms whereby these changes are conveyed to membrane ion channels have remained elusive.
Materials and methods: We have used different genetically modified mouse models and pharmacological tools in order to show that disruption of mitochondrial electron transport selectively abolishes O_2 modulation of Ca^{2+} channels and arterial hypoxic vasodilation.
Results: Inactivation of Ndufs2, Cox4i2 or Hif1α in adult mice resulted in selective abolition of vascular smooth muscle responsiveness to acute hypoxia, although the overall arterial structure was preserved. Precontracted rings of femoral artery from these genetically modified mouse models did not show the characteristic relaxation induced by hypoxia. In addition, the L-type Ca^{2+} channel inhibition, cytosolic calcium decrease, and increase in NADH autofluorescence observed in systemic VSM cells exposed to hypoxia also disappeared in MCI-, Cox4i2- or Hif1α-deficient myocytes. We have also demonstrated a direct modulation of L-type Ca^{2+} channel currents by intracellular NADH and H_2O_2 in freshly dispersed vascular smooth muscle cells.
Conclusion: Hif1α-mediated expression of specific MCIV subunit isoforms in systemic arterial myocytes makes their mitochondria highly sensitive to changes in O_2 tension. In hypoxia, slowdown of mitochondrial electron transport results in the production of signalling molecules (NADH and H_2O_2), with inhibition of Ca^{2+} channels and vasodilation.
Key words: hypoxic vasodilation, mitochondrial oxygen sensing, calcium channels

P2 Sex and menstrual cycle phase effects on cerebrovascular responses to a single food cold pressor test.

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Introduction/Objective: The cold pressor test (CPT) involves cold water immersion of upper or lower limb(s) to elicit sympathetic nervous system responses. This study aimed to assess sex and menstrual cycle phase differences in cerebrovascular responses to a single foot CPT in men and naturally cycling women.
Materials and methods: Cerebral and systemic hemodynamics and respiratory responses were assessed during

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a 3-minute CPT in 27 young healthy adults (14 men – 1 visit, 13 women – 2 visits: early-follicular and mid-luteal). Common carotid artery (CCA) diameter and flow, middle cerebral artery blood velocity (MCAv) and conductance (MCA_{CVC}), mean arterial pressure (MAP), and end-tidal carbon dioxide ($P_{ET}CO_2$) were averaged at baseline and over the final 30 seconds of each minute of the CPT protocol – baseline (BL), CPT1, CPT2, and CPT3. Two-way repeated measures ANOVAs analyzed time-sex interaction for men and women or time-phase interaction for women across menstrual phase.

Results: For both time-sex and time-phase ANOVAs, there were main effects of time on CCA diameter ($p<0.001$, $p<0.001$), MCA_{CVC} ($p<0.001$, $p<0.001$), MAP ($p<0.001$, $p<0.001$), and $P_{ET}CO_2$ ($p<0.001$, $p<0.001$). There was an interaction effect of time and sex for MAP ($p=0.039$) and no interaction effect of time and phase for any variables. Main effects of sex were found for CCA diameter ($p<0.001$), CCA flow ($p=0.007$), and MAP ($p=0.011$), with no main effect of phase across the menstrual cycle for any variables.

Conclusion: These findings indicate sex differences (during early follicular phase) are not altered or explained by menstrual cycle phase (within women) in carotid artery and cerebral hemodynamics during the CPT.

Key words: Cerebrovascular physiology, sex differences, menstrual phase differences

P3 T-type Ca^{2+} channels are needed for sinoatrial pacemaker function and atrioventricular conduction in zebrafish larvae

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Introduction/Objetives: In the adult mammalian heart, $I_{Ca,T}$ current carried by $Ca_v3.1$ channels plays a functional role in sinoatrial node automaticity, and in the atrioventricular (AV) node and Purkinje network. In zebrafish, $Ca_v3.1$ channels (encoded by the *cacna1g* gene) represent 63% and 55% of all Ca^{2+} channel transcripts in the atrium and ventricle, respectively, in contrast with the adult human heart, in which LTCCs ($Ca_v1.2$ ∝-subunit) represent 96-98% of the transcripts. The aim of this work was to characterize the functional relevance of TTCC in the larval zebrafish heart.

Materials and methods: We use a zebrafish transgenic line expressing in the heart the ratiometric Ca^{2+} biosensor Twitch-4, which allows simultaneous imaging of Ca^{2+} and contractility in the beating heart.

Results: We found that the TTCC blocker ML218 caused several cardiac alterations in 3 days-post fertilization larvae: 0.3 mM induced bradycardia and slow AV conduction with no effects on Ca^{2+} transient amplitude and contractility, whereas 1 mM produced AV blocks such as Mobitz type I and 2:1 AV block. Similar effects were found in *cacna1g* G0 knockout larvae generated by the CRISPR-Cas9 method: no altered contractility, AV blocks, slow AV conduction, and higher heart rate variability than wild-type larvae.

Conclusion: Our results show that TTCCs are involved in automaticity of the sinoatrial node and conduction in the AV canal in zebrafish larvae, as in mammals, and that Ca^{2+} influx through TTCCs does not play a major role in the contraction of the larval zebrafish heart, despite the reported abundance of TTCCs in atrial and ventricular cardiomyocytes.

Key words: Zebrafish, heart, T-type Ca^{2+} channels

P4 Sex-specific miR-125b-5p expression in myocardial infarction in Senescence-accelerated mice

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Introduction/Objectives: miR-125b-5p has been proposed as regulator of biological processes as cell death or Inflammation, with controversial implication on the myocardial infarction. Our previous data suggested a possible involvement of sex and aging in miR-125b-5p expression. We determined the expression of miR-125b-5p and its predicted target genes in heart tissue in an experimental acute myocardial infarction (AMI) model in male and female senescence-accelerated mice (SAM), and its dependence on estradiol in cultured endothelial cells.

Materials and methods: AMI was surgically induced by coronary artery ligation in six-month-old SAM-Prone (SAMP8, n=24) mice and its control SAM-Resistance (SAMR1, n=24) (Ethics 2020/VSC/PEA/0128). Sham-operated mice were used as control. Mice were euthanized 4 hours after surgery. Human Umbilical Vein Endothelial Cells (HUVEC, Lonza) were treated with physiological concentrations of 17β-estradiol (0.1 to 10 nM) for 24 h. miRNA targets were predicted and functional enrichment analysis was performed. RNA expression was evaluated by RT-qPCR.

Results: AMI down-regulated cardiac miR-125b-5p in all mice groups ($p<0.05$), except for female SAMP8 mice. Conversely, miR-125b-5p predicted targets, CDH5 (VE-cadherin) and ITGA9 (integrin-α9) which are related with angiogenesis and hypertrophic cardiomyopathy respectively, were overexpressed in cardiac tissue from all mice groups ($p<0.05$) except for SAMP8 females. In HUVEC, 17β-estradiol reduced miR-125b-5p and increased ITGA9 and CDH5 expression ($p<0.05$).

Conclusion: There is a sex-specific regulation by miR-125b-5p expression on genes related with cardiac remodeling in males and SAMR1 females, which is not observed in aged SAMP8 females. This supports an interaction between sex and aging in the molecular mechanisms of myocardial infarction.

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Key words: acute myocardial infarction (AMI); sex-differences; miR-125b-5p.

P5 PORH evokes a significant reperfusion in the contralateral forearm even without ischemia

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Introduction/Objetives: Post-occlusive Reactive Hyperemia (PORH) has been used to explore the microcirculatory

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response to ischemia in vivo. Recent data suggested that PORH mechanisms and physiological significance should be re-equalized. Here, we analyze the effects of PORH in the non-occluded contralateral limb.
Materials and methods: Fourteen male participants (48.0 ± 8.8 years old.) were selected. PORH was applied to one randomly chosen arm using a pressure cuff. Occlusion was maintained for 2 minutes with a 200 mmHg pressure. Perfusion was assessed throughout the procedure in both hands by laser Doppler flowmetry (LDF) and on the middle contralateral forearm by photoacoustic tomography (PAT). Cardiovascular function was followed by the Task Force platform.
Results: LDF measurements in baseline (Phase 1), during occlusion (Phase 2), and recovery (Phase 3) demonstrated that even if a "zero" perfusion is not achieved, this partial occlusion in one single arm evokes significant perfusion reductions in both limbs, followed by intense reperfusion when occlusion ceases. The associated blood movement back-and-forth between the superficial and deep skin plexus was clearly revealed by PAT. No other variables changes were observed except for mean arterial pressure.
Conclusion: Our data confirm that this reduction of flow and pressure in a major artery with PORH is not due to ischemia. Instead, the fast reperfusion following hypoxia and hypovolemia triggers a global cardiovascular adaptive response, not only a local vasodilation effect, also observable in the contralateral limb. The potential utility of this reflex as a global indicator of human cardiovascular adaptation should be explored.
Key words: PORH; contralateral limb effects; perfusion and cardiovascular adaptation; laser Doppler flowmetry; photoacoustic tomography.

P6 Nesfatin-1 Alleviates Cardiovascular Injury and Improves Impaired Aortic Contractility in Ovariectomized Rats: Role of Vagal Afferents

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Introduction/Objetives: The hormonal changes during menopause negatively affect female cardiovascular health. Expression of nesfatin-1 (NES) in hypothalamus varies by estrus cycle stages. This study aimed to investigate therapeutic potential of NES on menopause-induced cardiovascular alterations and involvement of vagal afferents in NES-induced protective effects.
Materials and methods: Under anesthesia, female Sprague-Dawley rats underwent either ovariectomy (OVX; n=12), sham-surgery (n=12) or OVX+vagal afferent denervation with perivagal capsaicin (1%; OVX+VAD; n=12). Starting by 16th day of recovery, rats were injected subcutaneously with NES (0.2µg/kg/day) or saline for 25 days until they were euthanized. Thoracic aorta contraction/relaxation (KCl/carbachol) responses were studied in isolated organ bath. Antioxidant superoxide dismutase, catalase, glutathione levels were measured in heart tissues and microscopic damage in Hematoxylin-Eosin-stained aortae and heart was evaluated. Statistical analysis was made using one-way ANOVA.
Results: Cardiac catalase levels in saline-treated OVX ($p<0.05$) and OVX+VAD ($p<0.001$) groups were reduced when compared to sham-operated group, while NES-treatment replenished catalase levels in both OVX-groups ($p<0.05$). Similar but non-significant changes were observed in superoxide dismutase and glutathione levels. Detachment of endothelium and smooth muscle injury in aortae and disorganization of cardiomyocyte fibers were observed in saline-treated OVX-rats, while aortic and cardiac morphology were normal in NES-treated OVX groups. Decreased aortic contraction-relaxation responses of saline-treated OVX-rats ($p<0.05$) were improved in NES-treated OVX-rats ($p<0.05$), but not in NES-treated OVX+VAD group.
Conclusion: Nesfatin-1 treatment alleviated menopause-induced cardiovascular injury and increased antioxidant enzymes. Moreover, impaired contraction-relaxation response of aorta in ovariectomized rats was improved by nesfatin-1 via activation of vagal afferent fibers.
Key words: Nesfatin, menopause, cardiovascular injury

P7 Effects of Fast-Paced Music Listening On Fat Oxidation Rate and Heart Rate Variability During Exercise

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Introduction/Objetives: Various interventions that increase sympathetic activity just like fast-paced music does, have been shown to reduce HRV (heart rate variability) and increase maximum fat oxidation (MFO) during exercise. This study aimed to examine the effect of fast-paced music listening on HRV and MFO during exercise in healthy individuals aged 18-25 by applying the FatMax test.
Materials and methods: The study was conducted with 14 male and 13 female subjects. Subjects were randomly performed the FatMax test with music followed by without music or in the reverse order. The test conducted in the morning, aimed to identify the exercise intensity that maximized the rate of fat oxidation. During the test, the quantities of oxygen (VO_2) consumed and carbon dioxide (VCO_2) in the exhaled air were measured using a mask, and the rate of fat oxidation was computed using the Frayn formula. MFO point was also examined. VO_2, RPE (rate of perceived exertion) and HR (heart rate) at that point were assessed. HRV time-domain (SDNN, rMSSD) and frequency-domain (VLF, LF, HF, LF/HF) parameters in 200 heartbeat were analyzed before, during, and after the test.
Results: MFO, VO_2 and RPE were compared using paired *t*-test, no statistically significant differences were found. HRV were compared using multivariate Anova test at rest, the point when MFO occurs during exercise and post-exercise periods. rMSSD and HF were significantly different between rest and post-exercise periods with music ($p<0,05$), but not significant without music.
Conclusion: In conclusion, our findings indicate that listening to fast-paced music affects the autonomic nervous system during physical activity.
Key words: Heart Rate Variability, Maximum Fat Oxidation, Music

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P8 Extracellular histones enter HUVEC cells and blood vessel organoids and colocalize with the autophagy mediator LC3B

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Introduction/Objetives: Sepsis, a widespread global ailment, involves an exaggerated immune response, leading to hyperinflammation and immunosuppression. Extracellular histones, released during hyperinflammation, significantly contribute to sepsis pathogenesis. The objective of this study is the dissection and analysis of the relationship between extracellular histones, cell membrane, and intracellular pathways related to endocytosis and autophagy, with the histone-induced cytotoxicity observed in the endothelium of septic processes.

Materials and methods: This study explores cytotoxic effects and cellular localization of histones, along with their colocalization with the autophagy mediator LC3B, in human umbilical vein endothelial cells (HUVECs) and blood vessel organoids, using confocal microscopy and flow citometry assays.

Results: Exposure to various doses of purified extracellular histones in both HUVECs cultures and organoids revealed sub-lethal doses leading to histone entry and interactions with LC3B, whereas high doses induced cytotoxicity. Incubating cells or organoids at low temperature before histone exposure prevented entry, abrogating colocalization with LC3B and cytotoxic effects.

Conclusion: In conclusion, this study offers insights into histones' cytotoxicity, cellular localization, and autophagy functional interactions, enhancing our understanding of the molecular bases of sepsis pathophysiology within HUVEC and blood vessel organoids. Sepsis, inflammation, vascular endotehlium

Key words: Sepsis, inflammation, vascular endotehlium

P9 Elevated miR-92a levels in patients with chronic coronary syndrome and concomitant diabetes compared to patients with chronic coronary syndrome without diabetes and healthy volunteers.

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Introduction/Objetives: There are factors that inhibit atherosclerotic plaque progression like KLF2, KLF4 (endothelial protectors), MertK (apoptotic debris clearance inhibitor) and IL-10 (anti-inflammatory cytokine) and they are downregulated by corresponding microRNA particles: miR-92a downregulates KLF2, miR-10b – KLF4, miR-126 – MertK, and miR-98 – IL-10. Diabetes type 2 is a disease developed due to insulin resistance which affects many pathways in cardiovascular system. The aim of the study is to check the difference in expression levels of aforementioned microRNAs in chronic coronary syndrome (CCS) patients with concomitant diabetes type 2 ("CCS DM" group) comparing to only CCS patients ("CCS noDM" group) and healthy volunteers ("HV" group).

Materials and methods: A population of n = 72 subjects – 20 CCS DM patients, 42 CCS noDM patients and 10 HV were enrolled. All CCS patients underwent coronary angiography which confirmed coronary stenosis in at least one coronary branch. The expression levels of microRNAs were assessed by quantitative reverse transcriptase real time PCR (qRT-PCR). Relative expression levels were compared by Mann-Whitney U Tests.

Results: The CCS DM patients presented significantly increased expression levels of miR-92a comparing to CCS noDM patients – median values 98.59 [55.56–159.07] vs 68.8 [40.67–97.93], p = 0.041. Similarly, CCS DM patients presented significanlty increased miR-92a expression levels comparing to HV - median values 98.59 [55.56–159.07] vs 66.82 [31.77–85.81], p = 0.044. No significant difference was found in comparison of miR-92a expression levels in CCS noDM and HV. Additionally, no significant differences were found between all the groups (i.e. CCS DM, CCS noDM and HV) in reference to other 3 examined microRNAs – miR-10b, miR-126 and miR-98.

Conclusion: Diabetes affects the expression levels of miR92a which downregulates KLF2 – a factor that protects againts atherogenesis.

Key words: microRNA, atherogenesis, diabetes

P10 Hypertension reduces endothelial NO production and activates VGCCs in aorta from females, but not from males.

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Introduction and objectives: Voltage-gated calcium channels (VGCCs) are involved in vascular smooth muscle contraction. Blockade of L-type VGCCs is used as an antihypertensive treatment and clinical studies show that blockade of L- and T-type VGCCs together may offer greater pharmacological benefits in the treatment of hypertension. Our aim was to determine whether the involvement of L- and T-type VGCCs in response to phenylephrine was increased in hypertension and affected by sex. Furthermore, we hypothesized that in the absence of NO the involvement of these channels would be increased.

Materials and methods: Aortic segments (3 mm) from four groups, spontaneously hypertensive female (SHRF, n=8), and

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male rats (SHRM, n=8) and their respective normotensive controls Wistar-Kyoto female (WKYF, n=8) and male rats (WKYM, n=8), were used. All at 16 weeks of age. Concentration-response curves to phenylephrine were performed in the absence of inhibitors, and in the presence of L-NAME (10^{-4}M) to inhibit NO synthesis, or verapamil (20μM), which at this concentration, blocks both L- and T-type VGCCs.

Results and conclusions: Aorta from SHRF group showed a higher vasoconstriction to phenylephrine compared to WKYF likely due to a lower NO production. In contrast, aorta from SHRM showed a lower vasoconstriction compared to WKYM with no changes in NO production. In the presence of verapamil, the phenylephrine curve was abolished in all groups. We conclude that hypertension decreases NO release and increases the involvement of VGCCs in response to phenylephrine in the aorta of females but not males.

Key words: Voltage-gated calcium channels, nitric oxide, aorta

P11 Intrinsic effects of a peptidase inhibitor, lbq657, on myocardial heterogeneity produced by ventricular local stretch

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Introduction/Objectives: Overactivation of Ca2+/calmodulin-kinase II, due to mechanical overload, can hyperphosphorylate the ryanodine receptor, to produce diastolic calcium release, triggering ventricular arrhythmias. Mechanical overload occurs in heart failure (HF); in which there is a premature sympathetic and the renin-angiotensin-aldosterone system, as well as the natriuretic peptide system, activation. To maintain the levels of the natriuretic peptides released, by plasma peptidases inhibition, and preventing the effects the angiotensin II release, the use of sacubitril-valsartan has been proposed. We have investigated the administration of peptidases inhibitor, LBQ657, on the myocardial heterogeneity due to local ventricular stretch.

Materials and methods: We have used 11 isolated and perfused rabbit hearts, in a Langendorff system, submitted to ventricular fibrillation (VF), maintaining the coronary circulation. A multielectrode was placed for ventricular recording and another for ventricular stimulation and to trigger VF to make a spectral analysis of the arrhythmia and to determine spectral concentration (SC), inversely related with myocardial heterogeneity and defined as the spectral energy in a window centered on the dominant frequency±1 Hz. Determinations were performed pre and during stretch (three min with an L-shaped device), in basal and in treatment with LBQ657 (5nM, 50nM and 0,5μM). An Anova (repeated measures) was applied. Significance when $p<0,05$.

Results SC decreased during stretch (3 min) in basal condition (24±5 vs 18.9±3) but not in 5 nM and 0,5 μM concentration of LBQ657, decreasing at 50 nM (24.7±6 vs 19.1±3).

Conclusion: LBQ657 could contribute to explain the protecting effects of sacubitril-valsartan in HF, decreasing the electrophysiological intrinsic heterogeneity of myocardium.

Key words: Myocardial stretch, electrophysiology, sacubitril

P12 Effects of valsartan plus the peptidase inhibitor lbq657, on the electrical activation complexity produced by myocardial stretch

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Introduction/Objectives: Acute ventricular stretch causes arrhythmogenic effects through mechanoelectric feedback that occurs in pathophysiological processes such as heart failure (HF). The use of the sacubitril-valsartan (SV) association was proposed as a therapeutic measure to maintain the levels of the beneficial natriuretic peptides released in HF, through sacubitril, and preventing the effects the angiotensin II release, through valsartan. We have investigated the effects of valsartan plus the active metabolite of sacubitril, LBQ657, (SV), on the intrinsic electrical activation complexity modifications during ventricular fibrillation (VF) produced by myocardial local stretch.

Materials and methods: Eleven isolated rabbit hearts perfused in a Langendorff and submitted to VF were studied. Epicardial electrical mapping techniques were used, applying a multielectrode plate for ventricular recording, and one electrode for ventricular stimulation and to trigger VF. An analysis was carried out using epicardial electrical mapping of each of the activation times that allows identify simple fronts, reentries, breakthroughs, etc. Complexity index was calculated using a formula that takes into account the amount and type of activations. Determinations were performed pre and during stretch (three min, with an L-shaped device), in basal and after treatment with SV: V (1μM) + LBQ657 (0,5 μM). An Anova (repeated measures) was applied. Significance when $p<0,05$.

Results: Stretching increased the complexity of activation at basal condition (1.32±0.4 vs 1.75±0.2), and after SV treatment (1.31±0.3 vs 1.72±0.2); Values are expressed in arbitrary units of complexity.

Conclusion: SV does not seem to exert direct effects on the modifications in the activation complexity produced by local ventricular stretch.

Key words: Myocardial local stretch, sacubitril, valsartan

P13 Carnosine Supplementation Restores Flow-Mediated Dilation via NRF2 Signalling Pathway in Middle Cerebral Arteries of Sprague-Dawley Rats on a High Salt Diet

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Introduction/Objectives: Carnosine (a beta-alanine+histidine dipeptide) exerts antioxidative effects in various diseases. High salt (HS) diet suppressed antioxidative mechanisms in microcirculation and subsequently impaired vasodilation affecting NRF2-signalling pathway. Present study aimed to determine the effects of carnosine supplementation on the flow-induced dilation (FID) in isolated middle cerebral arteries (MCAs) of Sprague-Dawley rats on low salt (LS) and HS diet.
Materials and methods: Sprague-Dawley rats 8-9 weeks old (N=8-9 per group) were fed LS diet (0.4% NaCl in rodent chow) or HS diet (4% NaCl), LS+CAR (oral carnosine supplementation, 150 mg/kg/day) and HS+CAR group (HS diet and carnosine) for 7 days, water ad libidum. Anesthetized rats (ketamine-chloride (75 mg/kg) and midazolam (0.5 mg/kg)) were decapitated. MCAs were isolated, mounted on glass pipettes and pressurized on a pressure myograph (DMT, Denmark). FID was determined by stepwise increases in pressure gradients (Δ10-Δ100 mmHg) in the absence/presence of NRF2 antagonist, ML385 (5x10-6 mol/L). The endothelium-independent vasodilation was tested with NO donor sodium nitroprusside (SNP; 10-6 mol/L). Gene expression of NRF2 and target gene NQO1 was performed with RT-qPCR. Statistical analysis: Two-way ANOVA; significance level $p<0.05$. All experiments conformed to EU Directive 86/609 and were approved by the local and national Ethical Committees.
Results: Attenuated FID in HS group was restored in HS+CAR group. NRF2 antagonist significantly attenuated restored FID in HS+CAR group, as well as in other groups. SNP-induced vasodilation was preserved in all groups. Carnosine significantly increased NRF2 and NQO1 genes expression.
Conclusion: Carnosine exhibits protective effect on FID in HS diet via NRF2 signaling pathway.
Acknowledgements: This study was supported by the Faculty of Medicine Osijek Institutional grantIP1-2022-MEFOS and IP26-2023-MEFOS and IP18-2024-MEFOS (for all PI Ines Drenjančević) and IP16-2022-MEFOS and IP22-2023-MEFOS (PI Zrinka Mihaljević).
Key words: flow-induced dilation, microcirculation, carnosine

P14 MitoKATP Channels Couple Penile Blood Flow to Metabolism: The Role of Nitric Oxide

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Introduction: MitoKATP channels are involved in cardiac protection against ischemia and oxidative stress. Erection is a neurovascular process that occurs via vasodilation of penile arteries and relaxation of erectile tissue, mediated by NO released from nerves and vascular endothelium.
Objectives: The objective of this study was to determine the role of mitoKATP channels in the regulation of penile arterial tone and mitochondrial bioenergetics.
Materials and methods: The effects of the mitoKATP channel activator BMS191095 (BMS) were evaluated on rat penile arteries mounted on microvascular myographs. Mitochondrial bioenergetics were measured by Seahorse XF Analyzer. ROS levels were measured by MitoSOX and AmplexRed techniques.
Results: BMS induced potent relaxations that were significantly reduced in endotheliumdenuded arteries, in the presence of L-NOARG, as well as with LY294002. The vasodilatory response to exogenous NO was inhibited by the sarcolemmal KATP channel blocker glibenclamide, the mitoKATP channel blocker 5-HD, and the BKCa blocker iberiotoxin. Bioenergetic parameters showed a decrease in basal respiration, oxygen consumption for ATP synthesis, and glycolytic activity after BMS treatment.
Inhibition of endogenous NO synthesis reversed the inhibitory effects of BMS on these parameters but revealed a NO-independent inhibitory effect on maximum respiration and spare respiratory capacity. Exogenous NO had dual inhibitory-stimulatory effects on mitochondrial respiration. BMS reduced mitochondrial ROS levels in both the presence and absence of L-NOARG.
Conclusion: This study demonstrates that activation of mitoKATP channels induces endotheliumdependent penile arterial vasodilation via the PI3K/Akt/NO pathway and reduces mitochondrial respiration and ROS production through NO-dependent and independent mechanisms thus coupling blood flow and metabolism.
Key words: mitoKATP channels, NO, penile arteries
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P15 Carnosine oral supplementation improves hypoxia-induced dilation in Sprague-Dawley rats on a high-salt diet

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Introduction/Objectives: 7-day high salt (HS) intake impairs micro- and macrovascular function due to increased oxidative stress. This study investigated whether carnosine as a "scavenger" of ROS (CAR; β-alanyl-L-histidine) affects hypoxia-induced dilation (HIR) and whether the possible mechanism involves the NRF2 signaling pathway.
Materials and methods: Healthy Sprague-Dawley rats, both sexes, 8-10 weeks old were divided into four groups (6 rats per group) – CTRL (control group, 0.4% NaCl), HS (4% NaCl in diet for 7 days), CTRL+CAR (oral carnosine supplementation, 150 mg/kg/day for 7 days) and HS+CAR group (HS and carnosine supplementation). HIR was studied in isolated norepinephrine-precontracted aortic rings (Isolated Organ Bath (Experimetria LTD)) in the presence or absence of NRF2 inhibitor (ML-385, 5x10-6 mol/L). mRNA expression of superoxide dismutases 2 and 3 – SOD2 and 3, glutathione-peroxidases 1 and 4 – GPx1 and 4, and catalase – CAT, in aortic tissue was assessed by RT-qPCR. All experimental procedures conformed to the European Guidelines (directive 86/609) and were approved by the local and national Ethical Committees (#2158-61-46-23-36, EP355/2022).
Results: HIR was impaired in the HS group and restored in HS+CAR. NRF2 inhibitor suppressed relaxation to hypoxia in HS and HS+CAR groups. The relative expression of SOD2 was significantly increased in the CAR groups compared to

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CTRL and HS groups; expression of GPx1 and GPx4 was increased in the HS+CAR group compared to the HS group, and there was no difference in SOD3 and CAT expression.
Conclusion: Carnosine upregulates antioxidant enzymes, restores HIR possibly via NRF2 signaling pathway.
Acknowledgments: This study was supported by the Faculty of Medicine Osijek Institutional grants IP16-2022-MEFOS and IP22-2023-MEFOS (PI Zrinka Mihaljević) and IP1-2022-MEFOS and IP26-2023-MEFOS and IP18-2024-MEFOS (PI Ines Drenjančević)
Key words: high salt, carnosine, hypoxia-induced relaxation

P16 Luteolin alleviates metabolic dysfunction-associated fatty liver disease induced by a high-fat diet in rats

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Introduction: Metabolic dysfunction-associated fatty liver disease, or MAFLD, has emerged as a major public health concern worldwide. Luteolin, a natural flavonoid, has been shown to possess several-beneficial biological properties.
Objectives: This study aims to evaluate the effect of luteolin and the probable mechanism on MAFLD in high-fat diet (HFD) fed rats.
Methods: Male Sprague-Dawley rats were fed with a high-fat diet with 15% fructose in drinking water for 12 weeks to induce metabolic syndrome and MAFLD. After that, HFD-fed rats were given either luteolin (50 or 100 mg/kg/day) or metformin (100 mg/kg/day) as treatment for four weeks.
Results: Luteolin reduced blood pressure in a dose-dependent manner in metabolic syndrome rats compared to untreated control animals ($p< 0.05$). Luteolin also improved metabolic parameters induced by HFD since it decreased body weight, fasting blood glucose, serum insulin, cholesterol and triglyceride ($p< 0.05$). Additionally, HFD-fed rats had elevated levels of plasma alanine phosphatase (ALT), which was reduced by luteolin treatment ($p<0.05$). HFD-fed rats showed hepatic steatosis, the accumulation of hepatic lipid droplets, which was alleviated by luteolin treatment ($p< 0.05$). Furthermore, luteolin significantly reduced malondialdehyde (MDA) in plasma and hepatic tissue and raised plasma catalase activity in HFD-fed rats ($p< 0.05$). Metformin was a positive control and had a comparable impact on hepatic steatosis and metabolic syndrome as luteolin did in HFD-fed rats.
Conclusion: These findings revealed that luteolin ameliorated HFD-induced MAFLD in rats, possibly via reducing signs of metabolic syndrome and oxidative stress in HFD-fed rats.
Key word: Metabolic dysfunction-associated fatty liver disease; Luteolin; High-fat diet; Oxidative stress

P17 Mediating role of extracellular vesicles in hypertensive retinal damage

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Introduction/Objetives: Our group has identified oxidative alterations in the retina of mice with arterial hypertension (AH) induced by the administration of L-NAME, a nitric oxide synthesis inhibitor. Additionally, we have demonstrated that extracellular vesicles (EVs) can modulate the production of reactive oxygen species (ROS) in hypertensive-like retinal pigment epithelial cells. These findings suggest that EVs play a mediating role in hypertensive ocular damage.
Materials and methods: In this study, four groups of male C57B/6J mice were used: 1) normotensive mice; 2) mice with AH induced by L-NAME intake for 6 weeks; 3) normotensive mice subjected to intravitreal (IV) injection of EVs isolated from the plasma of hypertensive mice; 4) hypertensive mice intravitreally injected with EVs from normotensive animals. The IV injections of EVs were performed on days 1, 3, and 5 after L-NAME treatment, where applicable; groups 1 and 2 were injected with PBS vehicle (Sham). Intraocular pressure (IOP) was recorded before and after IV injections. Animals were euthanized on day 7 post-injection, and the eyeballs were extracted to measure ROS in the retina using dihydroethidium (DHE) staining and to assess the activity of NADPH oxidase (NOX), a major source of superoxide anion production. Immunohistofluorescence of NOX isoforms and glial fibrillary acidic protein (GFAP) were also performed in paraffin-embedded retinal sections.
Results: EVs from normotensive and hypertensive animals showed a similar appearance, with an average size of 150-200 nm. IOP was increased in hypertensive animals, and this alteration was counteracted by the injection of EVs isolated from normotensive mice. Conversely, IV injections of EVs from L-NAME-treated animals increased IOP in normotensive mice. This opposite pattern between EVs from normotensive vs. hypertensive mice was also observed in the analysis of ROS, NOX activity/localization, and GFAP expression following IV injections.
Conclusion: Our results suggest: 1) the role of EVs as transmitters of hypertensive retinal oxidative damage; 2) the potential importance of EVs in the prevention and/or treatment of ocular diseases.
Key words: extracellular vesicles, hypertension, NADPH oxidase, retina, ROS.

P18 Characterization of pro-angiogenic effect of blood sera from patients with breast cancer.

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Introduction/Objetives: The malignancy of breast tumors is characterized by the 'angiogenic switch', where the tumor acquires its own vascularization thanks to the stimulation of

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endothelial cells (EC) by the neoplastic inflammatory microenvironment. Here, we examined the pro-angiogenic effect of blood serums (BS) collected from 33 patients with breast cancer on HMEC-1 (Human microvascular endothelial cell line-1). We analyzed BS effects on the activation of Notch-1, CREB, and Orai1, a store-operated Ca2+ channel.
Materials and methods: HMEC-1 were stimulated with 5% BS from controls and patients. The ability to form vessel-like structures was measured by tube formation assay using Matrigel. Migration and proliferation were evaluated by wound healing assay and immunofluorescence, respectively. Protein and mRNA expression were analysed by western blot and qRT-PCR.
Results: Elisa assay confirms an increase of VEGF-A levels in the BS of breast cancer patients as compared to control. HMEC-1 stimulation with 5% of BS promotes tube formation, indicating that BS boosts vessels-like formation. Next, we explored the role of CREB in stimulated HMEC-1. CREB silencing (siRNA) inhibits different parameters of tube formation, and attenuates the expression of Dll-4 (Delta-like 4), NICD1 (Notch Intracellular Cleaved Domain) and Hes1, as well as it increases VEGFR2 (VEGF Receptor 2) and Orai1. Conversely, patient's BS was unable to enhance migration and proliferation of HMEC-1.
Conclusion: In conclusion, our data demonstrate that patients' BS activate tube formation of EC, involving CREB signaling pathway, which participate in the activation of Notch. The role of Orai1 in these processes seems not relevant.
Key words: Angiogenesis, breast cancer, orai1

P19 Characterization of the role of Nestin+ cells in fibrosis induced by myocardial infarction

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Introduction/Objectives: Ischemic heart disease is the leading cause of death worldwide. The ischemic event can result in an adverse cardiac remodeling (ACR) that may lead to irreversible heart failure. This ACR is attributed to various cellular populations as fibroblast and endothelial cells (EC) undergoing changes impacting the extracellular matrix, primarily through inflammation and fibrosis. Finally, this ACR distorts the myocardium and hinders the proper functioning of the heart. In the myocardium exist mesenchymal progenitor cells characterized by the expression of the protein NESTIN (Nestin+ cells). These cells have been extensively studied in different niches, highlighting their role in inflammation and fibrosis. Therefore, they may play a role in the ACR after an acute myocardial infarction (AMI). This study aims to characterize the role of Nestin+ cells in the regulation of fibrosis and inflammation, resulting from an AMI.
Materials and methods: We isolated and cultured Nestin+ myocardial cells and simulated the effects of an AMI and the subsequent revascularization using an in-vitro ischemia-reperfusion protocol. Moreover, we studied an AMI mice model.
Results: qPCR data obtained from in-vitro experiments and in vivo histological analyses of mouse hearts after an AMI demonstrate that *Nestin* expression increases after an ischemic event. Moreover, FACs and immunofluorescence analyses showed that Nestin+ cells co-express different cell type markers of fibroblasts and EC. All together, these data suggest that progenitor Nestin+ cells are stimulated after the ischemic event and seem to differentiate to fibroblasts and EC.
Conclusion: As conclusion of this study, Nestin+ cells may play a role in ACR after an AMI.
Key words: Adverse cardiac remodeling, *Nestin*, fibrosis

P20 Tissular alterations in the adult heart due to treatment with anthracyclines: role of autophagy and senescence

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Introduction/Objetives: Chemotherapy treated patients are at risk of cardiotoxicity, particularly ventricular dysfunction (DV-CTOX). Of these patients, 10-15% develop heart failure (HF) within ten years after surviving cancer.
This study aims to determine the alterations in the heart during anthracycline treatment.
Materials and methods: A cardiotoxicity induction model was performed using epirubicin in female Wistar rats (250g) randomized into two groups: CTOX group: Four intraperitoneal injections of 5 mg/kg epirubicin over four weeks, and Control group: rats infused with saline solution. Myocardial strain was evaluated by echocardiography at baseline, 2 weeks and post-treatment. Myocardial fibrosis was assessed using Masson's trichrome staining and western blot. Apoptosis was assessed by TUNEL and Annexin V assays. Autophagy and senescence were examined using western blot, β-galactosidase activity, and p16 by immunofluorescence.
Results: In CTOX animal model, we observed significant decrease in heart strain even before left ventricle ejection fraction (LVEF) reduction, indicating cardiac dysfunction. We observed an increase in fibrosis, and changes in the expression of Collagen I, TGFβ2, αSMA, and MMP1, indicating extracellular matrix remodelling. Interestingly, apoptosis cell death was not detected, however, we observed significant increase in β-Gal signal and p16 presence in nuclei. Autophagy changes included increased LC3B, with a positive correlation with left ventricle ejection fraction, and increased LC3BII/LC3BI ratio.
Conclusion: Epirubicin induces heart dysfunction associated with extracellular matrix changes, alterations in tissue homeostasis, and promoting the increase in sencesence and autophagy-related proteins
Key words: cardiotoxicity, autophagy, senescence

P21 Arterial stiffness assessed by Cardio-Ankle Vascular Index (CAVI) and its modified versions (kCAVI, $CAVI_0$, $kCAVI_0$) in obese adolescents with and without metabolic syndrome

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Introduction/Objetives: The arterial stiffness index CAVI (heart - *a.tibialis*) is increasingly used to evaluate early atherosclerotic changes. Several studies surprisingly found lower CAVI values in patients with obesity. Metabolic syndrome studies, despite showing increasing CAVI with increasing MS components, also found a negative association of CAVI with waist circumference or BMI. Alternative indices: kCAVI (heart - *a.poplitea*; includes smaller part of peripheral circulation), $CAVI_0$ (modified CAVI, supposed to be less dependent on blood pressure) and $kCAVI_0$ (blood pressure corrected kCAVI) describe arterial stiffness from different points of view. The aim of our study was to compare CAVI, kCAVI, $CAVI_0$, and $kCAVI_0$ between adolescents with obesity, adolescents with obesity + MS and normal-weight probands.

Methods: Indices CAVI and kCAVI were measured using VaSera VS-1500 (Fukuda Denshi, Japan) in 34 patients with obesity (17.08 ± 1.02 y., BMI: 35.47 ± 5.44 $kg.m^{-2}$), 12 patients with obesity and MS (17.37 ± 1.01 y., BMI: 36.38 ± 4.68 $kg.m^{-2}$) and 46 age-matched normal-weight probands (17.27 ± 1.01 y., BMI: 20.87 ± 1.70 $kg.m^{-2}$). $CAVI_0$ and $kCAVI_0$ were calculated from CAVI and kCAVI.

Results: CAVI, $CAVI_0$ and kCAVI were significantly lower in the group of obese patients compared to both control group and group of obese + MS patients, and in all these indices there was no significant difference between the group of obese + MS patients and control group. Only index $kCAVI_0$ was significantly higher in the group of obese + MS patients compared to both obese and control groups.

Conclusion: Only $kCAVI_0$ (in contrast to CAVI, kCAVI and $CAVI_0$) indicated increased arterial stiffness in the group of obese + MS adolescents compared to obese and control groups.

Grants: VEGA 1/0283/21

Key words: Cardio-Ankle Vascular Index, obesity, metabolic síndrome

P22 Nerolidol Mitigates Cardiac Damage Following Myocardial Ischemia-Reperfusion Injury in Rats

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Introduction/Objetives: Nerolidol, a naturally occurring sesquiterpene alcohol with a floral odor (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol), is known for its antioxidant and anti-inflammatory properties. These characteristics suggest that nerolidol may offer cardioprotective benefits against myocardial ischemia-reperfusion injury (MIRI). However, no studies have yet investigated the protective effects of nerolidol against MIRI in vivo. This study aims to evaluate the impact of nerolidol on rats subjected to MIRI.

Materials and methods: To establish the animal models, myocardial ischemia was induced by occluding the left anterior descending coronary artery (LAD) for 60 minutes, followed by a reperfusion period of three hours in male Sprague-Dawley rats. Nerolidol or a vehicle control was administered intravenously 15 minutes before LAD occlusion. We assessed the severity of MIRI-induced arrhythmia, myocardial infarction, and the activity of lactate dehydrogenase (LDH) in plasma.

Results: Our findings indicate that nerolidol administration significantly reduced ventricular arrhythmia and mortality associated with MIRI. Furthermore, nerolidol decreased the size of myocardial infarcts and lowered plasma LDH activity following MIRI.

Conclusion: nerolidol exhibits a cardioprotective effect by mitigating the impact of myocardial ischemia-reperfusion injury.

P23 Empagliflozin reverts the changes produced by metabolic syndrome in the frequency-domain components of short-term heart rate variability. An experimental study

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Introduction/Objetives: Metabolic syndrome (MetS) has been linked to a higher prevalence of cardiac arrhythmias, being atrial fibrillation the most frequent, but the mechanisms are not well understood. One possible underlying mechanism may be the abnormal modulation of autonomic nervous system (ANS) activity, which can be quantified analyzing heart rate variability (HRV). In addition, the administration of selective sodium-glucose cotransporter type 2 (SGLT2) inhibitors, has been shown to reduce arrhythmogenic activity, but the mechanisms are currently unknown. We aimed to study the modifications induced by the administration of empagliflozin (an SGLT2-i) on short-term HRV, using an experimental model of diet-induced MetS.

Materials and methods: NZW rabbits were randomly assigned to Control (n=8), fed for 32 weeks with a standard rabbit diet; MetS-1 (n=10), fed for 32 weeks with a high-fat, high-sucrose diet (HFHS); or MetS-2 (n=6), fed for 32 weeks with HFHS and treated during the last 4 weeks with empagliflozin (1.5 mg/kg). Standard frequency-domain analyses were performed.

Results: We found a decrease in the very low frequency band in (p=0.013), and an increase in the low-frequency and high-frequency bands (p=0.012 and 0.026, respectively) in MetS-1 animals. These results may be indicative of ANS dysfunction and baroreflex alterations. No difference was found between Control and MetS-2 groups in any of the parameters studied.

Conclusion: Empagliflozin reverted the autonomic remodeling induced by MetS, which may have important implications for the risk of developing cardiac arrhythmias.

Key words: heart rate variability, sodium-glucose cotransporter-2 inhibition, empagliflozin, metabolic síndrome

P24 Inhibition of sodium-glucose cotransporter type 2 in metabolic syndrome: effects on time-domain and

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non-linear analysis of short-term heart rate variability

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Introduction/Objetives: Metabolic syndrome (MetS) has been linked to a higher prevalence of sudden cardiac death, but the exacts mechanisms are not completely known. Abnormal modulation of autonomic nervous system (ANS) activity may have an important role and can be quantified through the analyses of heart rate variability (HRV). On the other hand, the use of selective inhibitors of the sodium-glucose cotransporter type 2 (SGLT2) has shown protective effects against cardiovascular disease and cardiac arrhythmias. Our main objective was to analyze the effects of SGLT2 inhibition (empagliflozin) on short-term HRV, using an experimental model of diet-induced MetS.

Materials and methods: NZW rabbits were randomly assigned to Control (n=8), fed for 32 weeks with a standard rabbit diet; MetS-1 (n=10), fed for 32 weeks with a high-fat, high-sucrose diet (HFHS); or MetS-2 (n=6), fed for 32 weeks with HFHS and treated for the last 4 weeks with empagliflozin (1.5 mg/kg). At week 32, animals were anesthetized and 15-minute ECG recordings were used to analyze HRV. Standard time-domain and nonlinear HRV analyses were performed using Kubios®.

Results: Triangular index (TI) significantly decreased in MetS-1 (p=0.007) whereas no changes were found between Control and MetS-2 groups. There were no differences in the rest of time-domain or nonlinear parameters between groups.

Conclusion: MetS produced a decrease in TI, indicative of sympathovagal imbalance, which was reverted by SGLT2 inhibition. Thus, the use of SGLT2 inhibitors may decrease arrhythmogenesis through an indirect effect on ANS activity.

Key words: heart rate variability, sodium-glucose cotransporter type 2, empagliflozin, metabolic syndrome

P25 Role of H2O2 and NO in the regulation of renal vascular tone and mitocondrial Bioenergetics

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Introduction: Mitochondrial reactive oxygen species (mtROS) have traditionally been linked to vascular injury in metabolic pathologies (*Shah & Brownlee, Circ res., 2016*). However, recent studies show that mtROS play a key role as physiological regulators of cellular signalling mechanisms, and specifically in the vascular endothelium, whose energy demands are lower than those in vascular smooth muscle (VSM). (*Kadlec et al, Basic Res Cardiol., 2016*). Endothelial H2O2 is involved in the vasodilatation of renal arteries (*Muñoz et al, Free Radic. Biol Med., 2017*).

Objectives: The objective of this study was to assess the role of H2O2 and NO in renal vascular tone and mitochondrial bioenergetics.

Materials and methods: Arterial function of renal arteries was determined in microvascular myographs and
Mitochondrial bioenergetics were measured by SeahorseXF Analyzer. The effects of the endotelial agonists acetylcholine (ACh), the NO donor (SNAP) and exogenous H2O2, and the blocker of nitric oxide synthase L-NOARG, and the H2O2 scavenger, catalase, were evaluated in both renal arterial tone and mitochondrial bioenergetics.

Results: Exogenous H2O2 induced relaxations that were inhibited by L-NOARG and blocked by catalase.
Incubation with catalase enhanced basal mitochondrial respiration and oxygen consumption for ATP production, resulting in a reduction of the spare respiration capacity. SNAP increased the basal mitochondrial respiration and oxygen consumption for ATP production while L-NOARG reduced maximal respiration and spare respiratory capacity.

Conclusion: This study shows H2O2 induces vasodilatation linked to a reduction in mitocondrial respiration, while NO relaxed renal arteries but increased mitochondrial bioenergetics. These results suggest that vasodilator ROS may play a physiological role coupling renal blood flow and metabolism.

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Key words: *H2O2, NO, mitochondrial respiration, ROS, renal arteries*

P26 Protective action of empagliflozin against the modifications of short-term intrinsic pacemaker variability induced by metabolic syndrome. An experimental study in isolated rabbit heart

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Introduction/Objetives: Metabolic syndrome (MetS) is a condition associated with the presence of multiple non-communicable chronic diseases and has been linked with an increased risk of developing cardiac arrhythmias. Although the exact mechanism underlying these conditions is not fully understood, sinoatrial node dysfunction could be involved. Conversely, the use of selective inhibitors of the sodium-glucose cotransporter type 2 (SGLT2) has shown protective effects against cardiac arrhythmias. We aimed to evaluate the modifications induced by empagliflozin administration on intrinsic pacemaker variability in an experimental model of diet-induced MetS.

Materials and methods: NZW rabbits were randomly assigned to Control (n=19), fed for 32 weeks with a standard

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rabbit diet; MetS-1 (n=18), fed for 32 weeks with a high-fat, high-sucrose diet (HFHS); MetS-2 (n=6), fed for 32 weeks with HFHS and treated for the last 4 weeks with empagliflozin (1.5 mg/kg). We recorded 15 minutes of spontaneous cardiac activity in sinus rhythm. Standard HRV parameters in linear (time- and frequency-domain) and non-linear analyses were studied using Kubios®.
Results: We observed an increase in the minimum multiscale entropy (p=0.043) and a trend towards the increase (p=0.061) in the maximum multiscale entropy. The complexity index also increased in the MetS-1 group (p=0.029). No difference was found between Control and MetS-2 in any parameter.
Conclusion: Diet-induced MetS increased the complexity and irregularity of the intrinsic pacemaker activity in isolated heart, and thus not submitted to extrinsic nervous and humoral influences. The treatment with empagliflozin reverted these changes, which may be effective to decrease the risk of developing arrhythmias.
Key words: heart rate variability, sodium-glucose cotransporter type 2, empagliflozin, metabolic syndrome, sinoatrial node

P27 Dimethyl fumarate prevented the development of chronic crowding stress-induced hypertension by reducing vascular contractility

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Introduction/Objectives: This study investigated the effects of chronic social stress and dimethyl fumarate (DMF) in borderline hypertensive rats, focusing on the nuclear transcription factor (erythroid 2-derived 2)-like 2 (NRF2) gene *Nfe2l2* and selected NFR2-regulated antioxidant and inflammatory genes in the heart and on vascular function in the femoral artery.
Materials and methods: Rats were exposed to chronic crowding stress, DMF treatment (30 mg/kg/day), or a combination of both for six weeks. Blood pressure was measured non-invasively, gene expressions by RT-qPCR and vascular function by measuring noradrenaline (NA)-induced vasoconstriction and endothelium-dependent and independent relaxations by wire myography.
Results: Chronic stress increased BP, *Nfe2l2* expression and NA-induced vasoconstriction, though it did not affect relaxation responses and the left heart ventricle-to-body weight (LHV/BW) ratio. DMF elevated *Nfe2l2* and NRF2-target gene expressions in the heart and the LHV/BW ratio, supposedly due to reductive stress induced by continuous NRF2 activation, but did not alter BP and vascular functions. When combined with stress, DMF treatment prevented stress-induced hypertension and mitigated NA-induced vasoconstriction without altering relaxation functions. In addition, the combination of stress and DMF increased expressions of inflammatory markers *Tnf* and *Nos2*.
Conclusion: This study suggests that DMF can prevent chronic stress-induced hypertension by reducing vascular contractility. However, DMF itself may produce negative effects on the heart and induce inflammatory processes when combined with chronic crowding stress. This indicates a need for careful consideration of long-term DMF treatment, considering its impact on the heart.
The study was supported by VEGA-2/0157/21 and APVV-22-0296.
Key words: gene expressions, vascular function, heart hypertrophy

P28 Effect of monomethyl fumarate to vascular functions in rats exposed to acute stress

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Objectives: This study investigated the effects of the monomethyl fumarate (MMF, activator of nuclear transcription factor NRF2 function) in acute air-jet stress-exposed borderline hypertensive rats, focusing on changes in vascular function and nitric oxide (NO).
Material and Methods: Rats were divided into control group, MMF-treated (8 mg /kg i.v.) group, rats exposed to 3-times repeated acute air-jet stress during the 140-min period and MMF-and-stress co-exposed rats. Vascular function was assessed in the femoral arteries by wire myograph. NO synthase (NOS) activity was measured by conversion of 3H-L-arginine. NOS inhibitors W1400 and Nω-nitro L-arginine methyl ester (L-NAME) were used to investigate inducible and endothelial NOS's participation in contraction/relaxation.
Results: Air-jet stress significantly reduced NO production in the aorta vs. control without changes in the other three groups. This was associated with the significant increase in noradrenaline (NA)-induced contractions in the stress group vs. the remaining three groups of rats while no differences were in ACh-induced relaxations among the groups. W1400 significantly reduced NA-induced contractions in the stress group, but not in the remaining three groups of rats. W1400 reduced sensitivity to ACh similarly in all groups. Preincubation with L-NAME significantly increased NA-induced contractions and reduced sensitivity to ACh in all groups of rats to approximately the same levels.
Conclusion: MMF failed to alter vascular function in the control condition but significantly reduced NA-induced contractions in acute air-jet stress conditions, suggesting its acute antistress effects.
The study was supported by VEGA 2/0157/21 and MVTS-CA-20121.
Key words: vascular function, nitric oxide, acute stress

P29 The effect of sex and perivasular adipose tissue on vasoactive responses in a prediabetic rat model

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Introduction/Objetives: The activity of perivascular adipose tissue (PVAT), a specific adipose tissue deposit surrounding blood vessels, may contribute to sex differences in vascular control. The aim of this study was to evaluate the relationship

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among the sex, PVAT and vascular function in Wistar and Hereditary Hypertriglyceridemic (HTG) rats.
Materials and methods: Vasoactive responses of the isolated thoracic aorta with preserved or removed PVAT were measured in adult male and female Wistar and HTG rats, and the contribution of nitric oxide (NO), hydrogen sulfide (H2S) and the inflammatory signaling pathways to endothelial function and contractility was monitored in females.
Results: HTG rats were hypertensive compared to Wistar rats, but females less than males. Increased 2-hour glycemia was observed in HTG rats compared to Wistar rats regardless of sex, however, HTG females revealed better glucose utilization than males. Females, regardless of sex, had better preserved endothelial function compared to males, and PVAT inhibited endothelium-dependent relaxation in all rats except HTG females. In HTG males, the pathologically increased aortic contractility compared to Wistar males was noted, on the other hand, in HTG females, the contractile responses were smaller thus approaching physiological level. In HTG females, NO contributed to endothelial function to a lesser extent than in controls, but the presence of PVAT eliminated this difference, which was consistent with the reduced expression of endothelial NO-synthase in aortic tissue but not in PVAT. Although the increased expression of several pro-inflammatory factors (TNFα, IL-6, iNOS) was confirmed in PVAT of HTG females, the level of monocyte chemoattractant protein-1, a factor contributing to the development of endothelial dysfunction, was decreased. Moreover, in HTG females, regardless of PVAT, endogenous H2S revealed beneficial anticontractile effect, which was associated with increased protein expression of H2S producing enzymes in both aortic and PVAT tissue. Moreover, unlike in controls, H2S produced by PVAT did not inhibit endothelium-dependent relaxation.
Conclusion: Despite increased inflammation, female HTG rats trigger protective vasoactive mechanisms associated with milder hypertension and insulin resistance, improved endothelial function and contractility which are linked to PVAT activity and include stimulation of sulfide and nitroso signaling. Supported by SK-CZ-RD-21-0102.
Key words: HTG, sex, vasoactivity, PVAT

P30 Cardiovascular effects of diosmetin in renovascular hypertensive rats.

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Background: Cardiovascular dysfunction and remodelling have been documented to be associated with hypertension. Diosmetin is a flavonoid glycoside isolated from citrus seeds and has a variety of biological properties.
Objectives: To examine the effects of diosmetin on cardiovascular function and hypertrophy in two kidney-one clips (2K-1C)-induced hypertensive rats.
Methods: Male Sprague-Dawley rats were divided into five groups (n=8/each): Sham + vehicle, 2K-1C + vehicle, 2K-1C + diosmetin (20 mg/kg), 2K-1C + diosmetin (40 mg/kg), and 2K-1C + telmisartan (5 mg/kg). Hypertension was created by clipping the left renal artery with a silver clip, whereas sham-operated rats had the same surgery without the clip. After three weeks of the induction periods, diosmetin, telmisartan or vehicle were orally administered daily for the final four weeks.
Results: Diosmetin (20 and 40 mg/kg) significantly decreased blood pressure in a dose-dependent manner in 2K-1C rats compared to untreated rats ($p<0.05$). It improved vasorelaxation induced by acetylcholine in aortic rings and mesenteric vascular beds. Enhancement of vasoconstriction responses to electrical field stimulation (EFS) in 2K-1C rats was suppressed by diosmetin treatment compared to untreated group ($p<0.05$). Reduction of ejection fraction and fractional shortening was observed in hypertensive rats and these were restored by diosmetin treatment. Diosmetin alleviated left ventricular and aortic hypertrophy in hypertensive rats ($p<0.05$). Increases in serum angiotensin-converting enzyme activity, plasma angiotensin II levels, and the angiotensin II type 1 receptor/TGF-β1 expression were suppressed by diosmetin treatment ($p<0.05$). Telmisartan was a positive control agent. It produced a similar effect on cardiovascular changes as diosmetin.
Conclusion: Diosmetin exerted an antihypertensive property and alleviated cardiovascular dysfunction and hypertrophy in hypertensive rats. These effects could be attributed to its capacity to reduce ACE activity and Ang II/AT1R/TGF-β signaling pathway.
Key word: Diosmetin, cardiovascular dysfunction and hypertrophy, Renin-angiotensin system.

P31 Impact of prediabetes on cardiovascular biomarkers, cardiac oxidative stress and cardiac remodeling in rats

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Introduction/Objetives: This study investigated the relationship between prediabetes and cardiovascular biomarkers, cardiac tissue oxidative stress, and metabolic changes. It also evaluated the influence of prediabetes on cardiac remodeling by assessing matrix metalloproteinases (MMP) -2 and -9 activities, and histomorphometric and immunohistochemical parameters of cardiac and pancreatic tissues.
Materials and methods: Male Wistar albino rats were divided into control and prediabetes groups induced by low-dose streptozotocin (STZ) injections (8 mg/kg daily for 5 days, ip). Serum cardiometabolic biomarkers were measured. Cardiac oxidative stress parameters (catalase, superoxide dismutase, malondialdehyde – MDA) and activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were assessed spectrophotometrically. LDH and MDH isoforms and MMP activities were evaluated electrophoretically. Histomorphometric and immunohistochemical analyses were performed on cardiac and pancreatic tissues.
Results: Prediabetic rats exhibited significantly higher homocysteine, glucose, insulin and HOMA-IR, along with an altered lipid profile and increased aspartate/alanine aminotransferase (AST/ALT) ratio compared to controls. Prediabetes induced oxidative stress, evidenced by elevated

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MDA levels. LDH and MDH isoform activities were altered, and MMP-2 and MMP-9 activities increased. Cardiomyocyte diameter increased, and PCNA (proliferating cell nuclear antigen) expression decreased in prediabetic rats. Immunohistochemical staining for insulin revealed more intense brown discoloration of the islets of Langerhans in the prediabetic group compared to controls.
Conclusion: Prediabetes in rats led to dysregulated glucose metabolism, altered lipid profile, and increased oxidative stress, along with histomorphometric changes in cardiac tissue. These findings suggest a link between prediabetes and cardiovascular risk, emphasizing early intervention strategies to prevent progression to diabetes and mitigate cardiovascular complications.
Key words: prediabetes, oxidative stress, cardiac remodeling

P32 Modifications in the structure of the ventricular heart tissue in a rabbit model of diet-induced metabolic syndrome

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Introduction/Objetives: Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors that produce both functional and structural alterations in the heart. Myocardial fibrosis may be a potent contributor to the impairment of the left ventricle (LV) systolic and diastolic function. For this reason, our objective was to describe the histological changes in ventricular tissue through the quantification of type I collagen (COL-I) in an experimental model of diet-induced MetS in rabbits
Materials and methods: 10 male NZW rabbits were randomly assigned to either the control (n=5) or MetS (n=5) groups and fed with a diet rich in fat and sugar for 28 weeks. Samples of tissue were taken from the left and right ventricles (RV) after the 28 weeks. COL-I in the epicardium, myocardium, and endocardium was measured using Picrosirius Red (45 images per animal). The Fast Fourier Transform was used to examine the organization of collagen fibers. Statistical analysis was performed using an unpaired t-test.
Results: A significant increase in COL-I was observed in the myocardium (p=0.014) and endocardium (p=0.001) of the RV in MetS group. In addition, COL-I increased in the epicardium (p=0.003), myocardium (p=0.001) and endocardium (p=0.003) of the medial anterior zone of the septum (MAT), and in the myocardium (p=0.007) and endocardium (p=0.001) of the basal anterior septum (BAT). COL-I organization also increased in the MAT area in MetS animals (p=0.028).
Conclusion: A MetS produced an increase in COL-I in both ventricles, and a more organized pattern in LV, which may have implications in arrhythmogenesis.
Key words: metabolic syndrome, fibrosis, ventricular tissue

P33 Diet-induced metabolic syndrome in rabbits results in structural alterations of the atrial heart tissue.

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Introduction/Objetives: Sustained obesity, hypertension, and metabolic syndrome (MetS) culminate in an abnormal atrial substrate that is characterized by structural remodeling, atrial fibrosis, and electrical impulse conduction problems. This defective atrial substrate is comparable to what is seen in several other diseases that predispose to atrial fibrillation. Therefore, our objective was to describe the histological changes in atrial tissue through the quantification of type I collagen (COL-I) in an experimental model of diet-induced MS in rabbits.
Materials and methods: 10 male rabbits (NZW) were randomized into a control (n=5) or MetS (n=5) group, fed for 28 weeks with a high-fat and high-sucrose diet. After 28 weeks, tissue samples were obtained from the right and left atria (RA and LA). Picrosirius Red was used for the quantification of COL-I in epicardium, myocardium and endocardium (45 photographs per animal). The organization of collagen fibers was analyzed with the Fast Fourier Transform. Statistical analysis was performed using an unpaired t-test
Results: A significant increase in COL-I was found in the epicardium (p=0.01) and myocardium (p=0.02) of the LA and in the endocardium (p=0.025) of the RA in the MetS group. Regarding the organization of collagen fibers, a more organized pattern of COL-I was found in LA of MetS animals (p=0.024).
Conclusion: Both atria undergo structural changes in MetS, as shown by an increase in type I collagen and its organization. The processes involved in the initiation and maintenance of arrhythmic and fibrillatory activity may be significantly influenced by these alterations.
Key words: metabolic syndrome, fibrosis, atrial tissue

P34 Young tobacco smokers show early subclinical circulatory modifications

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Introduction/Objetives: Cigarette smoking is a recognized risk factor for cardiovascular disease. However, early indicators of damage without clinical signs of impairment are still poorly documented. Our study investigated the adaptive responses of a suprasystolic reactive hyperemia maneuver in young smokers and compared it to a group of non-smokers.
Materials and methods: The study included 73 healthy volunteers (33 smokers, 40 non-smokers, mean age 20.8±2.8 y.o.) of both sexes, with similar anthropometric data. The Post-Occlusive Reactive Hyperemia (PORH) was applied on a randomly chosen arm in each participant, using a pressure cuff. The brachial artery occlusion was obtained with 200 mmHg pressure maintained inside the cuff for 2 minutes. Blood pressure was measured with a sphygmomanometer before, immediately after, and 10 minutes after the PORH maneuver to calculate the Mean Arterial Pressure (MAP). Perfusion was measured using LDF sensors on both hands (ventral side of the ring finger). The Smoke Index was calculated to characterize the intensity of smoking habits.
Results: Perfusion recovery after occlusion was significantly faster in non-smokers (***$p<0.0001$) compared to smokers suggesting that the microcirculatory adaptation after PORH is slower in smokers. This response was also significant ($p< 0.05$) in the contralateral arm, although less intense.
Conclusion: These results indicate that young, smokers already show early signs of hemodynamical impairment in the absence of any other signs or symptoms and suggesting an interesting path for further investigation.
Key words: Cigarette smoking; Post Occlusive Hyperemia Reactive (PORH); Microcirculation, Tobacco.

P35 The effect of dimethyl fumarate on heart and vascular responses of borderline hypertensive rats

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Introduction/Objetives: The nuclear factor NRF2 is a transcription factor regulating cellular homeostasis and several cytoprotective pathways. The study investigated the effect of NRF2 activator, dimethyl fumarate (DMF), on the cardiovascular functions of borderline hypertensive rats (BHR).
Materials and methods: Adult BHR male rats were divided into a control (Cont) and DMF-treated group (DMF, 30 mg/kg/day) for 6 weeks. Blood pressure (BP) and heart rate were measured using tail-cuff plethysmography. Nitric oxide synthase (NOS) activity was determined in left heart ventricle (LHV) and aorta tissue using the conversion of [3H]-L-arginine. The conjugated dienes (CD) concentration was determined spectrophotometrically at 233 nm. The gene expression was determined using RT-qPCR. Isolated segments of femoral and mesenteric arteries were studied with Mulvany–Halpern isometric myograph.
Results: DMF had no effect on BP but increased the LHV relative mass and NOS activity in LHV. Plasmatic concentrations of LDH and αHBDH were significantly increased. CD concentration in LHV was significantly reduced in line with significantly increased gene expressions of Nfe2l2, Hmox1, Sod1 and Gpx4. However, DMF had no effect on the endothelium-dependent and independent relaxations and noradrenaline, serotonin and K+- induced contractions of the femoral and mesenteric arteries.
Conclusion: Our results show that DMF significantly elevates relative LHV mass, the gene expression of Nfe2l2 and its selected target genes and reduces CD in LHV, however it does not affect vascular functions of BHR animals. Our results suggest a negative effect of long-term activation of NRF2 function on the heart due to induction of a hyperreductive state.
Key words: NRF2, dimethyl fumarate, cardiovascular system

P36 Lysosomal TRPML1 triggers global Ca^{2+} signals and nitric oxide release in human cerebrovascular endothelial cells

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Introduction/Objetives: The transient receptor potential mucolipin 1 channel (TRPM1) is an important Ca^{2+}-permeable, non-selective cation channel primarily localized on the membrane of lysosomes, which controls many functions such as vesicle trafficking, autophagy, and gene expression. Surprisingly, little information is available on the role of TRPML1 in vascular endothelial cells. The aim of the present study was to investigate whether TRPML1 induces Ca^{2+}-dependent nitric oxide (NO) release in the human cerebrovascular endothelial cell line hCMEC/D3.
Materials and methods: Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and NO production were measured by microfluorimetric techniques by loading the hCMEC/D3 with Fura-2/AM and DAF-FM, respectively. TRPML1 expression was evaluated by immunoblotting and immunofluorescence.
Results: Lysosomal TRPML1 is expressed and induces global Ca^{2+} signaling in hCMEC/D3 cells. TRPML1-mediated Ca^{2+} signals are triggered by lysosomal Ca^{2+} release and maintained by extracellular Ca^{2+} entry. In addition, TRPML1-mediated lysosomal Ca^{2+} release triggers endoplasmic reticulum (ER) Ca^{2+} release through inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$), leading to store operated Ca^{2+} entry (SOCE) activation following the depletion of the ER Ca^{2+} pool. The endogenous TRPML1 ligand phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) induced a global increase in $[Ca^{2+}]_i$ similar to that induced by TRPML1 activation. Lysosomal TRPML1 induced a Ca^{2+}-dependent massive NO production in hCMEC/D3.
Conclusion: Lysosomal TRPML1 channel is expressed and mediates a global increase in $[Ca^{2+}]_i$ in hCMEC/D3 cells. Lysosomal Ca^{2+} release through TRPML1 is supported by ER Ca^{2+} mobilization through InsP3Rs and by SOCE. Physiologically, TRPML1 is gated by the lysosome-associated phosphoinositide $PI(3,5)P_2$ and may be involved in the regulation of cerebral blood flow by promoting endothelium-dependent NO release.

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Key words: TRPML1, Ca^{2+} signaling, endothelial cells

P37 Alternative RAS pathway activation attenuates monocrotaline-induced pulmonary arterial hypertension

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Introduction: Pulmonary arterial hypertension (PAH) is a rare, chronic and debilitating disease characterized by increased pulmonary arterial pressure leading to right heart failure and – eventually death. Several drugs are approved for the treatment of PAH, but due to ongoing disease progression only half of the patients survive more than 7 years after diagnosis. A novel therapeutic approach based on pharmacologic activation of the alternative RAS was investigated in monocrotaline-induced PAH in rats.

Methods: PAH was induced in 12 week-old Wistar rats by an injection of monocrotaline (60 mg/kg). Immediately after PAH induction, the rats (n=11-12 in each group) were treated for 4 weeks with either a) vehicle, b) ALT01 (4.2 mg/kg per day), and c) the endothelin receptor antagonist bosentan (300 mg/kg/day) – representing the standard of care. Vehicle-treated sham rats served as healthy controls. Analysis included tissue and plasma equilibrium levels of angiotensin metabolites, echocardiography-determined right ventricular function and structure, pulmonary arterial pressure, systemic blood pressure and histomorphometrical evaluations.

Results: Ang II was significantly increased in lungs of monocrotaline treated animals concomitant with a profound increase in pulmonary arterial pressure (mPAP), reduced fractional shortening, dilated pulmonary valve diameter and a thickened and enlarged right ventricle. ALT01 treatment resulted in increased Ang 1 7 levels while Ang II levels were normalized. Bosentan treatment did not affect angiotensin peptide levels. The PAH induced increase in mPAP was almost completely abolished by ALT01 treatment, while systemic blood pressure remained unaffected. Right ventricular structure and function was effecti vely preserved under ALT01 treatment and pathological changes in fractional shortening (FS) were significantly reduced.

Conclusions: Small-molecule-induced pharmacologic modulation of the RAS might be a promising novel therapeutic approach in treating RAS-related diseases, including PAH. (Partly supported by VEGA 2/0025/23 and APVV-22-0264).

Key words: Monocrotaline, renin-angiotensin system, pulmonary hypertension

P38 The preglomerular vasculature damage and renal dysfunction in conditions of cardio-metabolic disorders

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Introduction/Objetives: Increased frequency of hypertension, insulin resistance, obesity and dyslipidemias has been reported to be associated with an enhanced risk of vascular damage, which induces metabolic, cardiovascular and renal diseases, and the onset of one conveys risk on the others through unclear mechanisms. The aim of this study was to investigate molecular mechanisms of renal damage and dysfunction in a fructose-induced metabolic syndrome

Materials and methods: We used male Wistar rats, divided into two groups (n=7): control and high-fructose drinking (FD) (35% fructose corn syrup for 16 weeks) groups. Metabolic disorders, changes of kidney, renal arteries and heart were studied by morphological, immunohistochemical, immunological and biochemical testing.

Results: All FD rats developed obesity, hyperglycemia, hypertriglyceridemia, increased levels of TNFa and uric acid in compared to the control group, as well as in the renal blood vessels amyloid deposits and renal damage (vacuolar degeneration in the proximal tubular epithelium) in the absence of evidence of renal dysfunction and electrolyte disturbances. FD group results showed endothelial dysfunction, early atherosclerosis in the renal arteries-increased SOD-1 and VCAM expressions and reduced NOS3 expression, features for left ventricular hypertrophy and necroptosis (significantlly higher RIP3 expression in myocardium and the coronaries) compared to the control.

Conclusion: Our results show that under conditions of fructose-induced metabolic disorders, early renal, vascular and heart impairment develops without any marked manifestation of renal dysfunction. Additional studies are required to determine the severity of each investigated marker for the future risk of worsening kidney function.

Key words: cardio-metabolic-renal disease, fructose

P39 Reduced oxygen consumption initiates cardioprotective stimulation of mitochondrial respiratory chain proteins.

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Introduction/Objetives: The state of reduced oxygen utilization in cardiac mitochondria induced by acute diabetes mellitus (D) shows a slowed down flow of electrons in the respiratory chain, resulting in a slowing of oxidative phosphorylation. In the initial phase of experimental diabetes, the cardioprotective response associated with the functional remodeling of mitochondria occurs. The aim was to determine whether proteomic changes of the mitochondrial respiratory chain are involved in adaptation processes under conditions induced by D.

Materials and methods: Isolated rat heart mitochondria (Wistar males) from the healthy control group (C) and the group with 8-day D (streptozotocin 65 mg/kg i.p.) were subjected to quantitative label-free LC-MS/MS proteomic analysis. The metabolic regulator dichloroacetate was administered (DCA, D+DCA) in two doses (150 and 75 mg/kg i.p.) 60 and 15 minutes before heart excision. The activity of

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respiratory chain complexes was determined spectrophotometrically.
Results: The obtained results showed stimulation of complex I (p=0,001) and complex II (p<0,001) and an increase in the activity of complex I (p=0,006) in the D group compared to the C. ATP-synthase expression was maintained. In the D+DCA group, we observed the maintenance of expression levels for complexes I, III, and V at the level of diabetic mitochondria, while complex II (p=0,002) and complex IV (p=0,035) were downregulated compared to the D group.
Conclusion: The achieved results of proteomic analyses indicate that the stimulation of the respiratory chain complexes I and II represents one of the compensatory mechanisms acting against the increased energy load induced by D.
This study was supported by: APVV-22-0264;APVV-19-0540;APVV-20-0242;VEGA2/0016/23.
Key words: mitochondria, proteomics, cardioprotective response

P40 The role of T-type VGCCs in the alpha1-adrenergic response varies depending on sex and is not affected by a high-fat and high-sucrose diet.

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Introduction and objectives: The high-fat, high-sucrose diet (HFSD) is commonly used to induce metabolic syndrome. Our aim was to study the role of T-type voltage-gated calcium channels (VGCC) on the alpha1-adrenergic response in the aorta of HFSD-fed rats and to analyse this focusing on sex.
Materials and methods: Aortic rings were isolated from 39-week-old Sprague-Dawley rats, which were divided into 4 groups: females (SD-F, n=8) and males (SD-M, n=9) fed a standard diet, and females (HFSD-F, n=8) and males (HFSD-M, n=9) fed a HFSD. Concentration-response curves to acetylcholine and sodium nitroprusside were performed to determine endothelium-dependent and -independent relaxation. Receptor non-specific and specific alpha1-adrenergic responses were studied. The latter in the presence and absence of $NiCl_2$ ($5x10^{-5}$ M), a T-type VGCC blocker. The results were analysed using GraphPad Prism 9 by applying a 2-way ANOVA.
Results and conclusion: The HFSD did not affect vasodilation in females, but induced a significant rightward shift of the concentration-response curve to acetylcholine in males without affecting the response to sodium nitroprusside. The HFSD did not affect the contractile response in females or males. Incubation with $NiCl_2$ ($5x10^{-5}$ M) shifted the phenylephrine curve to the right in females of both groups. However, in males, incubation with $NiCl_2$ had no effect. These results indicate that the HFSD induces aortic endothelial dysfunction only in males, and that T-type VGCCs are involved in the alpha1-adrenergic response selectively in the aorta of females, regardless of a HFSD.
Key words: High fat and sucrose diet, sex differences, T-type voltage-gated calcium channels.

P41 Interaction between platelet F11R/JAM-A and its immobilised counterpart switches the platelets adhering to fibrin from retracting to migrating phenotype

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Introduction/Objectives: F11Receptor/Junctional Adhesion Molecule-A (F11R/JAM-A) is a transmembrane protein which is expressed in blood platelets. It is also present in epithelial and in endothelial cells as a part of tight junctions. Platelet F11R/JAM-A facilitates adhesion under static conditions via homophilic dimerization and negatively regulates the activation of the αIIbβ3 integrin.
In recent years platelets were shown to adopt distinct phenotypes in thrombus and on the surface of inflamed vessel. Platelets located centrally in thrombi produce filopodia-like protrusions while those on the inflamed vascular wall are characterized by lamellipodia. Different phenotypes are possibly a manifestation of distinct functional roles of platelets in these conditions. The mechanisms which direct platelets to acquire specific phenotype are not fully understood.
The aim of the study was to verify whether the interaction between platelet F11R/JAM-A and surface-bound F11R/JAM-A may be involved in the switching of adhering platelets' phenotype.
Materials and methods: Fibrinogen-coated surface was treated with thrombin to form a fibrin-like mesh. Surfaces were additionally treated with recombinant F11R/JAM-A. PRP was incubated on prepared surfaces and adhered platelets were stained. Ilastik software, a machine learning tool for image analysis, was used to train a model recognising morphologically distinct populations of platelets: non-activated, filopodial and lamellipodial.
Results: Three distinct morphological types of platelets adhering to fibrin were equally distributed: non-activated 30,64 (22,04;43,31), filopodial 31,40 (22,59;55,23) and lamellipodial 27,2 (4,488;44,94) (median with IQR), while in the presence of F11R/JAM-A a shift of distribution towards lamellipodial form was observed: non-activated 16,39 (14,71;35,11) filopodial 20,89 (18,20;41,27) and lamellipodial 56,33 (16,61;64,97), p<0.05, n=10.
Conclusion: The interaction between platelet F11R/JAM-A and its surface-bound counterpart increased the fraction of lamellipodial platelets at the expense of non-activated and filopodial platelets. It suggests that F11R/JAM-A-dependent interactions are involved in switching of platelets' phenotype from that acquired in thrombus to the one characteristic in the inflammatory milieu.
Key words: blood platelets, F11R/JAM-A, fibrin, machine learning
This work was supported by the National Science Centre grant OPUS (UMO-2020/37/B/NZ3/00301)

P42 Correlation of electrocardiographic changes with echocardiographic assessment of right ventricular dimensions and function

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Introduction/Objetives: Physical activity leads to changes in heart ventricles, especially in the right one in endurance sports athletes, in the context of greater remodeling and increased wall stress. The aim of the study was to observe the connection of the changes in the electrical activity of the heart with the changes in its morphology and function

Materials and methods: Two non-invasive diagnostic methods for cardiovascular evaluation were utilized: electrocardiography (ECG) in 285subject (competitive athletes and amateurs) and 2D transthoracic echocardiography in 54 patients with ECG changes.

Results: There was a significant association of the occurrence of negative T-waves in anterior leads V1–V4 (r =
–0.271, p = 0.047) and in lateral leads D1, aVl, V5, V6 (r = –0.271, p = 0.047) in subjects with lower values of the displacement of the tricuspid annulus towards the apex of the right ventricle (RV) in systole (RVs'), while other significant associations with abnormal ECG were absent. The reduced RV deformation of the free wall and interventricular septum was statistically significantly associated with the more frequent presence of ST-segment changes (r = -0.291, p = 0.043), namely ST-segment elevation (r = –0.322, p = 0.0294) and increased Lyon-Sokolow index (r = –0.312, p = 0.029). 57. Athletes with a physiologically altered ECG had the lowest values of peak longitudinal strain in the reservoir phase (PALS), and the mean value was < 38%.

Conclusion: Electorcardiographic changes, especially abnormal ones, require additional investigations to detect potential cardiovascular diseases.

Key words: electrocardiography, echocardiography

P43 Effect of Sodium Valproate, a histone deacetylase inhibitor, on nitric oxide synthase and NO-dependent signaling in rabbits with acute myocardial infarction.

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Introduction and objectives: Studies have shown that severe heart conditions like myocardial infarction (AMI) can be linked to endothelial dysfunction, which is characterized by reduced NO bioavailability. Histone deacetylase (HDAC) inhibitors have been suggested as a potential treatment to regenerate myocardium and reduce ventricular injury after AMI. However, their impact on endothelial function is not yet fully understood. Therefore, this study examined whether sodium valproate (VPA), an HDAC inhibitor, protects endothelial function via nitric oxide (NO) synthesis and NO-dependent signalling in an in vivo model of AMI.

Material and methods: Twelve male New Zealand rabbits were subjected to occlusion of the left circumflex coronary artery for 1 h to induce AMI. The animals were distributed into three experimental groups: the sham-operated group (SHAM), the AMI group and the AMI + VPA group (AMI treated with VPA 500 mg/kg/day). After 5 weeks, blood samples and abdominal aorta were isolated to study the expression of proteins related to the synthesis and signalling of NO.

Results and conclusions: VPA tented to increase endothelial nitric oxide synthase (eNOS) expression after AMI, but did not affect phosphorylated eNOS, iNOS, or soluble guanylate cyclase. VPA did not reverse the increased arginase 2 isoform expression in AMI. The antioxidant key enzyme SOD1 was significantly downregulated in the AMI group ($P<0.05$ vs SHAM) and improved by VPA ($P<0.05$ vs AMI). VPA treatment exhibits an antioxidant effect after AMI, which could enhance the bioavailability of NO and restore AMI-induced endothelial dysfunction.

Key words: Sodium vaplroate, acute myocardial infarction, aorta

P44 Modulation of the Aryl Hydrocarbon Receptor Pathway by Rilpivirine in thoracic aorta and its perivascular fat from Standard and High-Fat Diet fed mice.

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Introduction/Objetives: Rilpivirine (RPV), a widely used anti-HIV drug, has demonstrated anti-inflammatory and antifibrotic properties in preclinical models of chronic liver diseases such as metabolic dysfunction-associated steatotic liver disease (MASLD). However, its vascular effects and whether its mechanism of action involves the aromatic hydrocarbon metabolism pathway remain unknown.

This work aimed to elucidate the effect of this drug on the gene expression of genes involved in the canonical Aryl hydrocarbon receptor (AhR) pathway in the thoracic aorta and its perivascular fat in a nutritional mouse model of MASLD induced by high-fat diet, and compare it with that of a standard diet.

Materials and methods: The animal study was approved by the University of Valencia Ethics Committee for Research and Animal Welfare. Female C57BL/6J mice aged ten weeks were randomly assigned to one of two groups: control group or vehicle and RPV treatment (5 mg/kg/day, p.o.) group during twelve weeks.

Results: Our findings showed that RPV treatment activates the canonical AhR pathway in both the thoracic aorta and perivascular fat, along with upregulation of Nrf2 gene expression, potentially promoting detoxification and antioxidant responses under a standard diet. However, in the context of a high-fat diet, RPV downregulates of the canonical AhR pathway in the thoracic aorta, while paradoxically leading to AhR overexpression in perivascular fat, accompanied by downregulation of other pathway-related genes.

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Conclusion: Our results suggest that RPV has the capacity to modulate AhR pathway gene expression but also underscore the influence of dietary fat percentage on these modulatory effects.
Key words: high-fat diet, Rilpivirine, aryl hydrocarbon receptor.

P45 Diosmin ameliorates vascular dysfunction and remodeling in hypertensive rats

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Introduction/Objetives: A flavone glycoside called diosmin is naturally present in the pericarps of several citrus fruits. Several studies suggest that diosmin has antioxidant and anti-inflammatory effects. Relatively little is known about how diosmin affects vascular alterations in hypertensive rats. The present study investigated the effect of diosmin on hemodynamic and vascular alterations as well as possible mechanisms in N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride (L-NAME)-induced hypertensive rats.
Materials and methods: Male Sprague-Dawley rats were given L-NAME (40 mg/kg, drinking water) and orally administered with diosmin (50 or 100 mg/kg) or azilsartan (5 mg/kg) for 5 weeks. The hemodynamics, vascular responses to vasoactive agents, aortic geometry, oxidative stress, inflammation and renin–angiotensin system (RAS) were investigated.
Results: Diosmin partially prevented the development of high blood pressure in rats treated with L-NAME ($p<0.05$). Diosmin improved endothelium-dependent vasorelaxation and suppressed contractile response to sympathetic nerve stimulation in aortic rings and the mesenteric beds. Hypertensive rats showed aortic remodeling including hypertrophy and collagen accumulation which was alleviated by diosmin treatment ($p<0.05$). Diosmin inhibited RAS activation, and oxidative stress parameters, and raised antioxidant enzymes and nitrate/nitrite production in hypertensive rats compared to the untreated group ($p<0.05$). Overexpression of angiotensin II receptor type I (AT1R), transforming growth factor beta 1 (TGF-B1), Janus kinase 2 (JAK2), signal transducer and activator of transcription 3 (STAT3), and matrix metalloproteinase-2 (MMP-2) proteins in aortic tissue was found in L-NAME treated rats and these were restored in diosmin treated group. Azilsartan, an angiotensin II receptor blocker, is a positive control agent. It had a comparable vasoprotective effect to that of diosmin.
Conclusion: These findings indicated that diosmin had a vasoprotective effect in hypertensive rats. The possible mechanism may involve the suppression of RAS and oxidative stress.
Key words: diosmin, hypertension, vascular remodeling

P46 Effects of the tea extracts ADM® White Tea and ADM® Tea Complex in angiotensin-II induced cardiovascular alterations in mice

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Introduction/Objetives: Tea consumption has been shown to exert beneficial cardiovascular effects due to its content of bioactive substances. The aim of this study was to evaluate the effects of supplementation with two tea extracts, one of white tea (ADM® White Tea: WT) and the other composed by a proprietary blend of black and green tea (ADM® Tea Com
Materials and methods: For this purpose, C57BL/6J male mice were fed for 8 weeks with a standard diet or a diet supplemented with 1.6% of WT or TC. After five weeks of treatment osmotic minipumps were implanted to infuse either AngII or saline for 28 days.
Results: AngII infusion was associated with heart hypertrophy, hypertension, endothelial dysfunction and overexpression of proinflammatory cytokines in aortic tissue. Supplementation of mice with both WT or TC reduced Ang-II induced endothelial dysfunction in aorta segments as well as the gene expression of the chemokine MCP-1 and the prooxidant enzymes NOX-4 and NOX-1 in the aorta. Moreover, supplementation with TC resulted in reduced systolic blood pressure and increased circulating levels of the vasodilator peptide Ang 1-7. In addition, mice supplemented with both extracts showed an overexpression of AT-2and of the anti-inflammatory cytokine IL-10 and a downregulation in the mRNA levels of AT-1. In the heart, supplementation with both tea extracts prevented ischemia-reperfusion induced apoptosis of cardiomyocytes through decreased expression of the proapoptotic caspase-8.
Conclusion: In conclusion, supplementation with WT or TC prevents cardiovascular alterations induced by AngII infusion both in the heart and in the aorta.
Key words: hypertension, endothelial dysfunction, apoptosis

P47 Evaluation of the possible beneficial effects of the postbiotic HTBPL1 on angiotensin II-induced cardiovascular alterations in mice.

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Introduction/Objetives: Hypertension is considered both a disease and one of the main important risk factor for the development of other cardiovascular diseases such as ictus or myocardial infarction. Supplementation with prebiotics and probiotics has shown beneficial effects for the treatment and/or prevention of some cardiovascular diseases including hypertension. However, supplementation with inanimate microorganisms and/or their components (postbiotics) is quite inexplored. Thus, the aim of this study is to assess the possible beneficial effects of a new postbiotic (ADM® HTBPL1) obtained from inactivated *Bifidobacterium* in the cardiovascular alterations induced by angiotensin II (AngII) infusion mice.
Materials and methods: For this purpose, C57BL/6J male mice were administered either excipient (maltodextrin) or ADM® HTBPL1 (10^{10} cells/mouse/day) in the drinking water. After five weeks of treatment osmotic minipumps were implanted to infuse either AngII or saline for 28 days.

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Results: AngII infusion was associated with heart hypertrophy, hypertension, endothelial dysfunction and overexpression of proinflammatory cytokines in aortic tissue. HTBPL1 supplementation reduced blood pressure and attenuated AngII-induced endothelial dysfunction in aortic segments. Moreover, mice supplemented with HTBPL1 showed decreased gene expression of the proinflammatory cytokine IL-6 and the prooxidant enzymes NOX-1 and NOX-4 as well as an overxpression of AT-2 and IL-10 in arterial tissue. In heart, HTBPL1 supplementation improved contractility after ischemia-reperfusion due to a decrease in cardiomyocyte apoptosis.
Conclusion: In conclusion, supplementation with the postbiotic HTBPL1 prevents endothelial dysfunction, lowers blood pressure and improves cardiac function afteer coronary ischemia-reperfusion in hypertensive mice.
Key words: angiotensin II, hypertension, cardiac function.

P48 Functional and ultrastructural modifications in aorta of rats with prolonged high fat diet and experimental-induced diabetes mellitus treated with *Cornus mas* L. extract

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Introduction/Objectives: Prolonged high fat diet (HFD) associated with diabetes mellitus (DM) induce endothelial dysfunction that might be mitigated by antioxidants' administration. The aim of this study was to evaluate the effects of *Cornus mas* L. extract on aorta wall in rats with HFD and experimental-induced DM.
Materials and Methods: Sprague Dawley rats fed for 9 months with HFD, the last month with experimental-induced DM and treatment, randomly allocated into five groups, received carboxymethylcellulose (CMC), insulin, pioglitazone, *Cornus mas* L. extract solution or gold nanoparticles phytoreduced with *Cornus mas* L. extract (AuNPsCM). At the end of the experiment, descending aorta rings were used to evaluate, in tissue bath, the responses to vasodilator (acetylcholine) and vasoconstrictor (phenylephrine) substances. Transmission electron microscopy (TEM) investigation was performed to analyse the ultrastructural aspects of aorta wall.
Results*:* *Cornus mas* L. extract simple solution enhanced significantly the aorta contractile and relaxation responses compared to control (CMC), insulin and AuNPsCM groups. TEM investigations showed aorta wall ultrastructure improved by *Cornus mas* L. simple solution and altered by AuNPsCM administration.
Conclusions: *Cornus mas* L. extract as simple solution ameliorated the ultrastructure and the responses of aorta wall while AuNPsCM solution produced aorta wall modifications and impacted this vessel's function.
Key words: endothelium, *Cornus mas*, diabetes mellitus

P49 Emerging risk factor in cardiovascular disorders: case of bisphenol S

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Introduction/Objetives: Cardiovascular diseases (CVDs) refer a range of conditions affecting the heart and blood vessels. Recent extensive evidence has shown that environmental exposure to endocrine disruptors (EEDs) can increase the risk of developing CVDs. In this context, little is known about the potential health hazards posed by bisphenol S, endocrine disruptor used in plastic materials, food packaging, food containers and paper receipts. It was detected in different physiological fluids. The objective of this study was to evaluate the impact of exposure to environmentally relevant concentrations of bisphenol S on the cardiovascular risk parameters of male Swiss albino mice during the post-lactation period.
Materials and methods: Male Swiss albino mice are exposed to Bisphenol S at different concentrations (25, 50, and 100 µg/kg/day, n=6/group) via their drinking water over a period of 10 weeks started after lactation period. Animals were treated in compleance with ethical guidelines for the use of animals in research. At the end of the treatment, animals were sacrificed, blood and hearts were collected for biochemical analysis of lipid profile and oxidative stress markers.
Results: Our results show that BPS can induce dyslipidemia and increase the atherogenic risk with induction of oxidative stress in cardiac tissues. These alterations are considered as key factors in cardiovascular disease induction and progression.
Conclusion: Postnatal exposure to Bisphenol S at low doses and under critical life windows present an emerging risk factor on human health in particular cardiovascular disorders.
Key words: Atherogenic index, Bisphenol S, cardiovascular disease, lipid profile, risk assessment

P50 Impaired REDOX balance and reduced mitochondrial bioenergetics in coronary arteries from obese rats

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Introduction/Objetives: Mitochondrial reactive oxygen species (mtROS) such as superoxide or hydrogen peroxide are produced during cellular respiration and regulate vascular tone (Kadlec AO, 2016; Windlansky ME, 2011). However, when cardiovascular risk factors like obesity are present, endothelial mitochondria produce excessive ROS, leading to vascular diseases (Guarini G, 2016; Tang X, 2014). In the present study we evaluated the involvement of mtROS and mitochondrial bioenergetics on the vascular function in coronary arteries from diet- induced obesity.
Materials and methods: Wistar rats were fed with high fat diet (HFD, 60%) or standard diet (STD). Coronary arteries were mounted in microvascular myographs and concentration-responses curves to acetylcholine (ACh) and serotonin (5-HT) were performed to assess vascular function. The involvement of mtROS in endothelial function was evaluated by the mitochondrial antioxidant MitoTempo. Also, mtROS levels were measured by fluorimetry using MitoSox ($O_2^{\bullet-}$) and

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AmplexRed (H2O2). Agilent Seahorse XF Pro analyzer was used to determine oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).
Results: Contractile response to 5-HT was increased, but endothelium-dependent relaxations to ACh were preserved in coronary arteries from obese rats. On the other hand, MitoTempo markedly reduced the ACh-mediated vasodilation in STD rats but not in HFD animals, indicating that mtROS-mediated relaxations were lost in obesity. Furthermore, mitochondrial O2•- production was higher in coronary arteries of HFD rats compared to STD controls and mitochondrial H2O2 levels were reduced in obesity. Finally, OCR and ECAR indicative of glycolytic activity were reduced in coronary arteries from HFD rats.
Conclusion: Despite endothelial vasodilatation is preserved in coronary arteries from HFD rats, our results suggest that increased mtROS levels are associated with alterations in mitochondrial function which might contribute to cardiovascular complications in obesity.
This work was supported by grants (PID2022-140536OB-I00 y PID2022-140536OB-I00) from Ministerio de Ciencia, Innovación y Universidades
Key words: mitochondria, oxidative stress, obesity, coronary arteries

P51 Obesity-induced REDOX imbalance and enhanced mitochondrial respiration contribute to renal vascular dysfunction

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Introduction/Objectives: Obesity is recognized as a risk factor for chronic kidney disease (CKD) independent of diabetes and hypertension (Wang, 2008; de Vries, 2014). Mitochondria produce energy, being the main source of reactive oxygen species (ROS). Increased mitochondrial ROS (mtROS) is linked to metabolic and vascular disorders (Forte, 2019). Kidney has high energy demands, relies on mitochondrial activity for ATP production. Recent studies show that mitochondrial dysfunction contributes to obesityrelated CKD (Zseto, 2016; Tang, 2016). The aim of this study was to assess the role of mitochondrial respiration and oxidative stress in kidney vascular dysfunction under obesity.
Materials and methods: Male Wistar rats were fed a high-fat diet (HFD) or a standard diet (STD). Renal endothelial function was evaluated using acetylcholine (ACh) to induce relaxation in preglomerular renal arteries, in the absence and after treatment with the mitochondrial antioxidant MitoTempo to assess mtROS involvement. mtROS production was measured with florescence by MitoSox and AmplexRed. Oxygen consumption ratio (OCR) and extracellular acidification rate (ECAR) were assessed using an Agilent Seahorse XF Pro analyzer.
Results: ACh-induced relaxation was impaired in HFD-renal arteries. Incubation with MitoTempo inhibited and improved endothelial relaxations in control and HFD arteries, respectively, suggesting mtROS production with vasoconstrictor effects in obesity. REDOX evaluation showed increased mtROS and decreased H2O2 levels in renal arteries of HFD animals. OCR was elevated in renal arteries of obese rats with augmented basal respiration, ATP production, maximal respiration, proton leak and no-mitochondrial respiration, that correlates with higher ECAR demonstrating increased in glycolytic metabolism.
Conclusions: These findings suggest a compensatory increase in mitochondrial respiration associated to altered REDOX balance in renal arteries from HFD. The latter results in overproduction of contractile mitochondrial superoxide (O2 -•) and decreased relaxing H2O2 that contribute to endothelial dysfunction in obesity-related kidney disease.
Keywords: Kidney disease, obesity, and mitochondria
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P52 SKF-96365 prevented Ang II induced IR injury via inhibition of TRPC1/3 channels in isolated rat heart.

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Introduction/Objectives: Members of the TRPC channel family are membrane proteins, which are expressed in most tissues and especially permeable to Ca^{2+} ions. The over-expression of TRPC channels in cardiomyocytes during ischemia/reperfusion (IR) injury are related to several heart diseases. However, studies on the effects of TRPC channel blockers on ischemic heart diseases are limited. Thus, we aimed to determine the effects of SKF-96365, a TRPC channel blocker, in IR injury, and to reveal the roles of TRPC channels in heart diseases, by applying it to healthy and ischemic hearts in Langendorff apparatus.
Materials and methods: In current study, 32 male *Wistar albino* rats, 3 months old and weighing between 250-300 g, were used. They were separated into four groups as control, IR, SKF-96365 (5 µM) and IR+SKF-96365. The hearts were attached to Langendorff isolated heart system and cardiodynamic parameters were recorded. Besides, at the end of experiment, hearts were homogenised for biochemical analysis, and all data were statistically evaluated.
Results: SKF-96365 administration ameliorated cardiodynamic parameters induced by IR injury in isolated rat hearts. A significantly increased levels of creatine kinase-MB, cardiac troponin T, myeloperoxidase, malondialdehyde and lactate dehydrogenase were determined in IR group, whereas SKF-96365 treated groups exhibited lower levels. Glutathione peroxidase and superoxide dismutase amounts were significantly lower in IR group, but higher in the SKF-96365 treated groups. Angiotensin II, TRPC1, TRPC3, TRPC6, and ENKUR levels significantly increased in IR group compared to the control group, while these parameters significantly decreased in the IR+SKF-96365 group compared to the IR group.
Conclusion: It was concluded that SKF-96365 partially have a cardioprotective effect, especially via inhibition of TRPC1 and TRPC3 channels and Ang II, on IR injury induced myocardial

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dysfunction. Also, because of decreasing the biochemical injury markers and increaseasing the antioxidant enzymes, TRPC channels seems to have important roles in the regulation of cardiac survival and functions.
Key words: TRPC, isolated heart, ischemia/reperfusion

P53 Impact of N-acetylcysteine on biochemical parameters in experimental model of myocardial infarction

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Introduction/Objectives: Myocardial infarction (MI) represents a significant global burden, ranking as the leading cause of morbidity and mortality within cardiovascular diseases. Following MI, prognosis is heavily influenced by various risk factors, such as hypertension. Hypertension demonstrably exacerbates the post-MI course by triggering the release of oxidative and inflammatory mediators within the heart tissue. The aim of the study was to determine the effects of hypertension and the antioxidant N-acetylcysteine (NAC) on biochemical and molecular parameters in an experimental model of myocardial infarction.
Materials and methods: Two groups of 12-week old SHR underwent MI by ligation of the left descending coronary artery and sham surgery. One group received NAC (25mg/kg/day) for 4 weeks prior to ligation. Seven days after surgery, proinflammatory cytokines were measured in plasma using Bioplex kit. Levels of nitric oxide synthase (NOS) activity, endothelial NOS (eNOS), inducible NOS (iNOS), and nuclear factor kappa B (NF-κB) protein expression were evaluated in the ischemic, border, and non-ischemic zones of the heart. Concentration of conjugated dienes, a marker of oxidative stress, was measured spectrophotometrically in the heart tissue.
Results: Our study revealed the most significant changes following MI occurred in the directly infarcted zone and the surrounding border zone. We observed an increase in harmful molecules reactive oxygen species (ROS) in these areas. Additionally, the total activity of NOS was decreased, while the expression of inducible NOS was increased. Antioxidant properties of NAC were confirmed by a decrease of conjugated diene concentration, a marker of oxidative stress. This reduction positively correlated with decreased NF-κB expression, molecule involved in inflammation as well as with reduction of cardiac fibrosis.
Conclusion: Preventive administration of NAC partially reverses the pathological consequences of MI by increasing total NOS activity, decreasing iNOS expression, reducing cardiac fibrosis and oxidative stress, and lowering inflammatory markers.
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Key words: myocardial infarction, N-acetylcysteine, nitric oxide

P54 High dose corticosteroid causes bradycardia in patients with multiple sclerosis

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Introduction: The use of high dose corticosteroids in MS exacerbations may cause cardiovascular adverse events, one of them is sinus bradycardia. Understanding the pathophysiology of corticosteroid induced bradycardia is paramount for healthcare providers.
Materials and methods: Patients who met the McDonald's criteria for definite MS and patients requiring admission for pulse steroid treatment with an acute recurrence were included. Individuals taking cardiac medications, or with a heart illness were excluded.Patients were given 1 g IV methylprednisolone in 2 hours for five to seven days in order to treat acute relapses. All patients had their electrocardiographs.
Results: We studied with 23 patients (6 males and 17 females, 26.1/73.9% respectively). The age of the patients was 34.6±9.9 (18-43) years and the duration of disease was 5.5±4.9 years.Most of the patients were relapsing–remitting MS in 73.9%.The most common cardiac arhythmia during corticosteroid pulse therapy was sinus bradycradia(n=6).
Conclusion: The combination of direct effects on cardiac myocytes, electrolyte disturbances, autonomic dysfunction, and individual genetic factors can contribute to the development of bradycardia in MS patients treated with high-dose methylprednisolone. Close monitoring and prompt intervention are crucial to manage this adverse effect and optimize patient safety.
Key words: multiple sclerosis, cardiac physiology, neurophysiology, bradicardia

Endocrinology, metabolism and nutrition

P55 Analysis of sleep architecture in metabolically healthy obese adult males: Understanding sleep health in obesity

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Introduction/Objectives: Obesity is associated with typical metabolic abnormalities such as insulin resistance, dyslipidemia, hypertension, and inflammation. However, a subset of obese population do not exhibit these metabolic abnormalities and are referred to as "metabolically healthy obese" (MHO). The relationship between obesity and poor sleep is bidirectional, with obesity contributing to sleep disturbances, and poor sleep quality potentially exacerbating obesity-related metabolic dysregulation. The study aimed to investigate sleep architecture in MHO adult males.
Materials and methods: This observational study was performed on 47 adult obese males who were grouped as metabolically healthy obese (MHO) based on their joint interim criteria (JIC). A whole night diagnostic polysomnography was done to study their sleep architecture.
Results: The average age and BMI of subjects was 39.51 ± 9.65 years, and 27.4 ± 2.25 kg/m2 respectively. The total sleep time (TST) was 304.3 ± 85.6 minutes and sleep latency was 14. 8 minutes ± 19.4 minutes. The participants had a REM latency of 141.5 minutes, mean N2% was 42.9 ± 13.5 minutes, N3% 18 minutes, and REM % 13.8 minutes. Their mean sleep efficiency was 77.17 ± 17.96 % and apnoea-hypopnoea index

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(AHI) was 25.14 events/hour. Positive correlation existed between total sleep time and sleep efficiency and a negative correlation of sleep latency was seen with their sleep efficiency. Participants had a positive correlation of their BMI with AHI so that AHI increases with increasing BMI.
Conclusion: The sleep disturbances are found in obese people even in the absence of metabolic abnormalities.
Key words: obesity, polysomnography, metabolic disorders

P56 Filamin A 16-24 C-terminal fragment modulates store-operated Ca^{2+} entry through increasing Orai1 and STIM1 expression.

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Store Operated Ca^{2+} Entry (SOCE) is a major mechanism for Ca^{2+} influx controlled by the intracellular Ca^{2+} stores. The stromal interaction molecule (STIM) protein family, with a predominant role of STIM1, activate Ca^{2+} channels located in the plasma membrane upon store depletion and allow Ca^{2+} entry through CRAC channels, mainly Orai1. Filamin A (FLNA), an actin-binding protein located in the cytoskeletal region of the membrane, has been demonstrated to play an essential role as a negative regulator of SOCE since it modulates Orai1-STIM1 interaction. We first found that FLNA protein is cleaved by calpain in HT-29 colorectal adenocarcinoma cell line, yielding a fragment containing repeats 16-24 ($FLNA^{16-24}$).
To further demonstrate the role of $FLNA^{16-24}$ as a regulator of SOCE, we expressed dsRED-tagged $FLNA^{16-24}$ in HEK-293 cells and analysed the expression of the key SOCE proteins. First, we confirmed the expression of dsRED-tagged $FLNA^{16-24}$ by Western blotting and confocal microscopy, and then observed that HEK-293 cells expressing $FLNA^{16-24}$ show increased Orai1 and STIM1 protein level without having any effect on the expression of Orai3 and STIM2. As a result, SOCE was enhanced in $FLNA^{16-24}$- expressing HEK-293 cells as compared to mock-treated controls. Finally, we inhibited protein synthesis treating cells with cycloheximide and confirmed that dsRED tagged $FLNA^{16-24}$-expressing cells still show an increased expression of Orai1 and STIM1, indicating that $FLNA^{16-24}$ fragment increase Orai1 and STIM1 half-life.
Conclusion: Altogether, these results explain how $FLNA^{16-24}$ fragment modulates SOCE and the expression of Orai1 and STIM1 proteins.
Key words: filamin A, Orai1, STIM1
Supported by: PID2022-136279NB-C21, PID2022136279NB-C22 (MCIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe"), and Junta de Extremadura-FEDER (IB20007 and GR18061).

P57 Orai1α mediates the agonist-induced NF-κB transcriptional activation in a PKCβ2 dependent manner

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Introduction/Objective: NF-kB is one of the main mammalian transcription factors involved at the inflammatory response but also in another physiological processes. The main activation pathway implies the interaction of cytokines, ROS or LPS, among others, but there are alternative pathways been one of it a calcium dependent pathway that involves SOCE, a calcium influx mechanism predominantly mediated by the calcium channel Orai1 and the endoplasmic reticulum calcium sensor STIM1. As in mammalian cells Orai1 present two variants, Orai1□ and a short form, Orai1□, we have investigated the functional role of both variants in NF-κB transcriptional activity.
Material and Methods: To assess carbachol-induced NF-kB activation we used a pNL3.2.NF-κB-RE[NlucP/NF-κB-RE/Hygro] Vector which codifies a luciferase which is activated when reacting with his substrate Nano-Glo® Luciferase Assay System WT or Orai1 knockout HEK-293 cells either transfected with Orai1α or Orai1β expression plasmids. Furthermore, we analyzed the Orai1-PKCβ interaction by immunoprecipitation and carbachol-induced calcium mobilization.
Results: The bioluminescence assay allow us to identify that Orai1alpha, but not Orai1beta, is capable to enhance the NF-kB transcriptional activity, despite there are not differences in carbachol-induced calcium mobilization in cells expressing Orai1□ or Orai1β. Furthermore, we found Orai1□-PKCβ2 interaction that cannot be reproduced with Orai1β due to the lack of the 63 N-terminal amino acids. This activation is a calcium-dependent mechanism in which Orai3 and TRPC1 are also involved.
Conclusion: Orai1α is capable to activate agonist-induced NF-kB activation by a mechanism dependent of calcium entry and PKCβ2 activation
Key words: Ca2+ influx, NF-κB, Orai1α
Supported by Agencia Estatal de Investigación PID2019-104084GB-C21/AEI/10.13039/501100011033 and Junta de Extremadura-Fondo Europeo de Desarrollo Regional (grants IB20007 and GR21008).

P58 Novel GPR120 agonist modulates systemic and neuroinflammation

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Introduction: Our aim was to target the systemic and central inflammation in obese mice by using a newly synthetized GPR120 analogue.
Materials & methods: We synthesized GPR120 agonist, called NKS5. We determined its molecular dynamic interaction with the receptor. In C57Bl/6 male mice, fed a high-fat diet, we elucidated its effects on obesity and its related parameters like systemic and neuroinflammation, by immunofluorescence staining, targeting microglia-specific calcium-binding proteins (IBA-1). Neuroinflammation severity was assessed in multiple hypothalamic, cortical and subcortical regions.
Results: Daily intake of NKS-5 via feeding bottles modulated gut microbiota, decreased food intake and progressive weight gain in obese, but not in control, mice. Interestingly, the NKS5 decreased circulatory proinflammatory factors such as lipopolysaccharide (LPS), tumor necrosis factor (TNF) alpha, and interleukin (IL) 6 in obese mice. Moreover, in intraperitoneal glucose tolerance test (IPGTT), the NKS5 accelerated the glucose clearance in obese mice. The NKS5

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also decreased the expression of IBA-1 expression in different regions of the brain.
Conclusions: Our results show that novel chemical GPR120 agonists might represent a new strategy to reduce systemic and neuroinflammation.
Key words: Fatty acids, inflammation, macrophages, lipids

P59 The nature of the CRAC cannel

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Store-operated calcium entry (SOCE) is a crucial mechanism for calcium influx regulated by the filling state of intracellular calcium stores and mediated by CRAC channels. This mechanism is essential for processes such as secretion or gene transcription, among others. Its main components are the proteins STIM and Orai. The STIM family components, STIM1 and STIM2, are located in the ER membrane and act as sensors of intraluminal calcium. Upon reduction in luminal ER calcium concentration, STIM proteins undergo a conformational change, interacting with and activating the Orai channels. Orai1 has two variants: Orai1α, the full-length form of 301 amino acids, and Orai1β, a short variant that arises from alternative translation starting from methionine 64 or 71. It has been shown that both variants have the ability to form channels that mediate SOCE; however, it is unknown if both variants can form a heteromeric CRAC channel.
Using molecular biology and biochemistry techniques, as well as fluorescence and super-resolution microscopy approaches, we demonstrate that at least a subpopulation of Orai1 variants associate to form part of the same complex and mediate CRAC. Our results in HEK-293 cells loaded with the fluorescent probe fura2 show that co-expression of a dominant negative of Orai1β significantly reduces calcium entry mediated by Orai1α. These results were confirmed using super-resolution microscopy techniques by studying variations in Geco-Orai1α fluorescence when exposed to calcium ions entering through the Orai1 channel. Finally, using FRET technique, we have been able to demonstrate an association between both isoforms after depletion of intracellular calcium stores.
Here, we present solid evidence supporting the idea that at least a subpopulation of Orai1 variants associate to mediate SOCE. Since the expression of both variants depends on cell type and an increase in the expression ratio of Orai1α/Orai1β has been observed in some diseases such as cancer, it is necessary to further investigate the processes that may regulate this heteromeric channel.
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Key words: Calcium homeostasis, Store Operated Calcium Entry (SOCE), Orai1α, Orai1β, FRET, Super-Resolution Microscopy.

P60 Cardiometabolic risk and Mediterranean diet adherence in the Cardiovascular Risk Prevention with a Mediterranean Dietary Pattern Reduced in Saturated Fat (CADIMED) Study

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Introduction/Objectives: The CADIMED study implemented a Mediterranean dietary pattern with complete elimination of red/processed meat, which have a less favourable saturated fat profile, compared with a control group receiving standard advice for cardiometabolic risk prevention. Here we calculated the cardiometabolic risk profile in association with adherence to Mediterranean diet in a baseline subsample of participants.
Materials and methods: CADIMED is a two-arm parallel randomized-controlled trial involving 156 adults with dyslipidemia (without pharmacological treatment), recruited from healthcare/community settings in Granada (Spain). The primary outcome will assess the impact on LDL-C and circulating palmitic acid after an 8-week dietary intervention compared with control. This study analysed 75 patients for whom we obtained data on diet, blood biochemistries and relevant cardiometabolic risk factors which were used to calculate the Framingham-REGICOR score, validated for the Spanish population, to estimate 10-year risk for cardiovascular disease (CVD) using age, sex, total cholesterol, HDL-C, smoking, systolic and diastolic blood pressure, and absence of type 2 diabetes. Mediterranean diet adherence was calculated through a 14-point Mediterranean Diet Adherence Screener (MEDAS) score and simple linear associations with cardiometabolic risk were explored.
Results: The analytic sample (n=75) comprised 55.3% men, with average age 52yr (SD 10.5), BMI 27 (SD 4.9). The average Framingham-REGICOR score was 2.7(SD 2.1), indicating a low CVD risk for this population. The average levels of other CVD risk factors were LDL-C 149.5 mg/dl (SD18.4); HDL-C 54.1 mg/dl (SD11.7); triglycerides 96.3 mg/dl (SD55); total cholesterol 222.8 mg/dL (SD23.9). The average adherence to the Mediterranean diet was low (MEDAS score 7.6 SD 2). There was no significant association between adherence and the Framingham-REGICOR score in this population.
Conclusion: Despite the lack of association between scores of Mediterranean diet adherence and cardiometabolic risk, the CADIMED intervention can still potentially benefit the lipid profile of this population following a Mediterranean diet approach without red/processed meat.
Key words: CVD, Mediterranean diet, SFA, LDL-cholesterol, Framingham-REGICOR.

P61 Cardiometabolic risk biomarkers associated to dietary patterns derived by reduced rank regression: a cross-sectional analysis of the Spanish pediatric cohort IBEROMICS

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Introduction/objectives: Diet plays a crucial role as a significant risk factor for childhood cardiometabolic disease, however there are few studies looking at overall dietary patterns (DP) and cardiometabolic disease in the Spanish pediatric population. We aimed to investigate associations between DPs derived by reduced-rank regression (RRR) with cardiometabolic biomarkers in the IBEROMICS pediatric cohort.
Materials/methods: We used data from Spanish children with overweight/obesity (n=862), including dietary, biochemical and body-composition data. Dietary data was obtained from two 24h-recalls. RRR models were used to derive DPs, using a total of 30 food groups and 3 response variables: total sugars, saturated fat, and dietary fiber density. We calculated adherence to each DP and investigated associations between DPs and cardiometabolic biomarkers using multivariable linear regression models.
Results: We found two DPs jointly explaining 43% of the total variance of the nutrients of concern. DP1 was characterized by a high intake of snacks, fresh fruit and sugary breakfast cereals, and a low intake of butter, margarine, olive oil and red/processed meat. DP2 was characterized by a high intake of whole-grain bread/pasta, sugary breakfast cereals and fresh fruits and vegetables and a low intake of butter, margarine, olive oil and ultraprocessed food. DP1 showed significantly negative associations with weight [β=-0,007, p=0.014] and fat mass [β=-0,001, p=0.006] in all children. Both DPs showed significant associations amongst pubertal children, DP1 was negatively associated with HOMA-index [β=-0,01, p=0.048] and DP2 negatively with fat mass [β=-0,015, p=0.027] and positively with QUICKI-index [β=5.004, p=0.049].
Conclusion: The study of whole DPs can provide valuable information to find combinations of foods that explain cardiometabolic risk in children.
Key words: cardiometabolic risk, childhood obesity, dietary pattern.

P62 High-phenolic cocoa diet boosts hippocampal neurogenesis in healthy adult mice without altering hippocampal neuroplasticity

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Introduction/Objetives: Cocoa, known for its health benefits due to its rich composition of minerals, fiber, methylxanthines, and polyphenols, particularly flavanols, lacks extensive exploration regarding its cognitive effects. This preclinical study aimed to investigate the effect of cocoa and cocoa polyphenols on hippocampal neuroplasticity and adult hippocampal neurogenesis.
Materials and methods: Young-adult C57BL/6JRj male and female mice were fed either a standard -control- diet (CTR) or a diet enriched with 10% of high-phenolic content cocoa (HPC) or low-phenolic content cocoa (LPC) for at least four weeks. Adult hippocampal neurogenesis and expression of brain derived neurotrophic factor (BDNF) in the hippocampus were evaluated using immunohistochemistry and western blot. Hippocampal synaptic response, long-term potentiation and presynaptic-dependent short-term plasticity were studied by electrophysiology.
Results: Both cocoa-enriched diets increased hippocampal BDNF expresión; however, only the HPC diet enhanced adult hippocampal neurogenesis, promoting the proliferation, survival and number of young adult-born neurons. Hippocampal electrophysiology revealed no alterations in neuroplasticity across diets.
Conclusion: Overall, HPC-cocoa enriched diet showed greater potential for neuroplastic benefits, highlighting the role of cocoa flavanol in dietary interventions aimed at improving brain health.
Key words: Phenolic content, Synaptic plasticity, Adult hippocampal neurogenesis

P63 In Vivo evaluation of cinnamon bark and fenugreek seed extracts for reversing pancreatic β-Cell Dysfunction in type -2 diabetic rat model

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Introduction/Objetives: Obesity, a prevalent metabolic disorder characterized by the excessive accumulation of adipose tissue, is intricately linked to the development of insulin resistance and type-2 diabetes mellitus The pathophysiological connection between obesity and insulin resistance involves the disruption of the intracellular insulin signaling pathway, exacerbating glucose dysregulation and insulin insensitivity.Present study focuses on evaluating the potential efficacy of cinnamon bark and fenugreek seed extracts, both alone and in combination, as interventions to counteract high-fat diet-induced obesity, insulin resistance, and hyperglycemia.
Materials and methods: For this study, Albino rats (N=36), initially weighing between 100-150g, were housed at the Animal Research Center at the Department of Physiology, Government College University Faisalabad. These rats were categorized into six groups: NC (Negative control), PC (diabetic and untreated), STD (standard control; diabetic+ metformin), T1 (diabetic+ cinnamon bark extract), T2 (diabetic+ fenugreek seed extract), and T3 (diabetic+ combination of cinnamon bark and fenugreek seed extracts). Excluding the NC group, all groups were subjected to a high-fat diet (30%) for a duration of 60 days to induce obesity. Diabetes was induced in all groups (excluding NC) through

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intraperitoneal injection of a low dose of streptozotocin (35mg/kg body weight). After supplantation of treatments for 21 days, all the rats were humanely euthanized, and both blood and pancreatic tissue samples were collected. Serum analyses encompassed glucose, insulin, and oxidative stress marker measurements. Gene expression analysis focused on MAFA, PDX1, NKX6.1, and NEUROD genes. Additionally, histopathological assessments were also performed to evaluate the changes in islets' architecture. Statistical analysis was conducted using one-way ANOVA via GraphPad Prism®.
Results: Results indicated that both extracts reviewed can potentially revert the high fat diet-induced obesity, hyperglycaemia, hyperinsulinemia, oxidative stress, and degenerative changes in the pancreas and promoted the beta cell regeneration changes indicated through improved PDX-1, MAFA, NKX6.1, and NGN3 expression levels.
Conclusion: Based on the results it is concluded that the synergistic properties of cinnamon and fenugreek seed extract hold promise for more effective therapeutic interventions in combating diabetes at its core.
Key words: Diabetes, Obesity, Natural products, Regeneration

P64 Effects of Apelin-13 on oxidative stress and auditory brainstem responses in STZ-induced diabetic rats

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Introduction/Objetives: Diabetes is a worldwide health problem and causes a variety of complications. Diabetes has many different complications, and one of them is hearing loss. Research on the relationship between diabetes and hearing loss has been ongoing for a long time. Apelin is typically expressed in vascular endothelium and adipose tissues, and Apelin-13 is the primary active isoform that specifically binds to the apelin receptor. The aim of this study is to investigate the effects of apelin-13 on electrocochleographic recordings, oxidative stress, and auditory brainstem responses in STZ-induced diabetic rats.
Materials and methods: Thirty-two male Wistar albino rats were divided into four groups: sham, diabetes, Apelin and Diabetes+Apelin. A single dose of 45 mg/kg ip STZ was administered to induce diabetes. Apelin groups received 50 µg/kg apelin-13 for seven days intraperitoneally. At the end of the Apelin and STZ applications, auditory brainstem responses (ABR) were evaluated. Later, cochlea tissue was removed and biochemical analyzes were performed.
Results: When compared to diabetic groups, Apelin therapy significantly improves the cochlea's total antioxidant status (TAS), while decreasing its total oxidant status (TOS) and oxidative stress index (OSI). Wave V latencies in diabetic groups were much longer than in control groups, according to measures of the auditory brainstem responses. Treatment with apelin partially reverses this effect, especially at certain frequencies and intensity levels.
Conclusion: Apelin may have a protective effect on the auditory system and may indicate a new course of therapy for the prevention and treatment of diabetic auditory neuropathy.
Key Words: Diabetes, Apelin, Auditory brainstem responses, Oxidative Stress

P65 Evaluation of the effect of eating attitude, determination the portion perception on anthropometric measurements

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Introduction: To eveluate the effect of portion perception on anthropometric measurements according to eating attitudes.
Materials and methods: This study was conducted with university students (116 female and 29 male). An eating attitude test (EAT-40) and the dietary intake frequency survey were applied. Afterthat photographs of selected foods (grilled meatballs, rice pilaf with tomato, chickpeas with meat, milk, bread and apple) in different portion sizes were shown to determine portion perception. Anthropometric measurements were taken with TanitaMC780.
Results: The mean body mass index (BMI) of individuals were found 21,2 kg/m^2 in women and 23,3 kg/m^2 in men. The mean fat percentage was found 24,1% in women and 17,8% in men. It was found that there was a significant difference between men and women in the body weight, BMI and fat percentage ($p<0.05$). When we analyzing eating disorders by gender, man with eating disorders are more than women. We found that 63.5% of those with eatimg disorders had a normal BMI. The percentage of individuals who responded correctly to one portion of grilled meatballs was significantly higher than those who did not have an eating disorder ($p<0.05$). When the general examination was performed; women's portion perception is better than men's.
Conclusion: We observed that assess how perception of portion sizes influences anthropometric measurements based on eating attitudes. This study, which lays the foundation for the belief that eating disorders arise from eating behaviors, sheds light on future studies.
Key words: Eating attitude, portion, portion perception

P66 Effects of mutated ATP synthase on cell physiology and mitochondrial deubiquitinases in treating mitochondrial diseases

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Introduction/Objectives: The severity of diseases caused by mutations in mitochondrial DNA (mtDNA) depends on the mutant-to-healthy mtDNA ratio, necessitating strategies to reduce mutant mtDNA. We hypothesize that inhibiting the mitochondrial deubiquitinase USP30, which removes ubiquitin tags from mitochondria, promotes degradation of dysfunctional mitochondria. We investigated the effects of the m.8993T>G mutation, which affects the ATP synthase, on cell physiology, and whether inhibiting USP30 promotes mitophagy and reduces mutant mtDNA.
Materials and methods: We treated 143B cybrid cells, containing wild-type or m.8993T>G mutant mtDNA, with the USP30 inhibitor MF-094 or solvent. We assessed cellular phenotype (proliferation, resazurin metabolism, ATP levels and extracellular acidification) under glycolytic and oxidative phosphorylation (OXPHOS) conditions. MF-094 bioactivity

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(mitochondrial ubiquitination and mitolysosome formation) and its effects on mutant mtDNA load were evaluated under glycolytic conditions.
Results: Under OXPHOS-dependent conditions, mutant cells showed decreased cell proliferation and ATP levels compared to wild-type cells. Under glycolytic conditions: mitochondrial inhibitors (rotenone, myxothiazol, antimycin and oligomycin) reduced resazurin metabolism in wild-type cells but not in mutant cells, which also exhibited increased extracellular acidification; MF-094 treatment increased TOM-20 ubiquitination and mitolysosome formation but did not alter mutant mtDNA levels.
Conclusion: The ATP synthase mutation led to high reliance on glycolysis. While MF-094 promoted mitophagy, it did not reduce mutant mtDNA levels under glycolytic conditions. We will next investigate whether mitochondria with mutant mtDNA are preferentially ubiquitinated under OXPHOS-dependent conditions – an effect that could potentially be enhanced by USP30 inhibition.
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Key words: Mitochondrial diseases, deubiquitinases, Mitophagy

P67 Differential expression of Orai1 variants in the lipid rafts domain of the plasma membrane

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Introduction: Orai1 is the key-pore forming subunit of the Ca^{2+} channels involved in the store-operated Ca^{2+} entry (SOCE). Two Orai1 variants have been identified, the mammalian-specific Orai1α (the full Orai1 protein) and Orai1β (lacking the first N-terminal 64-71aa). Although both variants can form the Ca^{2+} channel, the missed region in Orai1β contains several domains that give Orai1α and Orai1β different functional and regulatory properties, including a caveolin-binding domain (aa52-aa60) that could regulate the location of Orai1 in the lipid raft domains of the plasma membrane.
Objectives: To determine the expression profile of Orai1α and Orai1β in the lipid rafts in resting conditions and after the cell treatment with TG in HEK293-Orai1KO cells transfected with Orai1α-GFP or/and Orai1β -GFP.
Material and methods: We have performed a lipid raft isolation protocol in combination with Western blotting assay to detect the expression of each Orai1 variants in the isolated membrane fractions.
Results: When the Orai1 variants are expressed individually, Orai1α is predominantly expressed in the lipid rafts in resting conditions and after cell treatment with TG. Meanwhile Orai1β is expressed in both lipid rafts and non-lipid rafts fractions in resting conditions. Treatment with TG promotes the translocation of Orai1β to lipid rafts membrane fractions. Co-expression of both Orai1 variants promotes an increase of Orai1β expression in lipid rafts at rest and after treatment with TG, without affecting the expression profile of Orai1α.
Conclusions: Orai1α promotes the location of Orai1β in the lipid rafts in resting conditions and upon activation of SOCE.
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Key words: Orai1 variants, lipid rafts, SOCE

P68 Sex and age differences in bone architecture and bone marrow adipose tissue in tibiae of lean and diet-induced obese mice

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Introduction/Objectives: Osteoporosis is a comorbidity of obesity and aging which shows differences with the sex. In this study, we have investigated in mice, the differences in bone architecture and bone marrow adipose tissue (BMAT) content due to sex, aging and obesity.
Material and methods: Male and female C57BL6/J mice were fed with control diet (CD, 13% of fat) or high fat diet (HFD, 60% of fat) from 2 to 5 months-old (adult group) or from 2 to 11 months-old (aged group). Tibiae were fixed in 4% paraformaldehyde for 24 h. Bone micro-computed tomography (μCT) was used to study bone architecture. Next, 4% osmium stained and μCT were used to quantify the BMAT content.
Results: Trabecular bone percent (BV/TV %) and trabecular number (Tb.N) were higher in male compared to female mice in adult and aged groups. Trabecular space (Tb.S) was higher in adult female compared to male mice, whereas it was higher in aged male compared to female. In both adult and aged mice, HFD decreased BV/TV % and Tb.N in male, without modifying it in female mice. HFD did not modified Tb.S. In adult mice, BMAT was higher in female than male, and HFD increased BMAT content in both sexes. However, in aged mice, HFD only caused significant increase on BMAT in female mice.
Conclusion: Bone architecture and BMAT content are influenced by sex, aging and obesity. This highlights the importance of considering the mice sex when designing experimental protocols for bone metabolic studies.
Key words: Bone, BMAT, obesity, sexual dimorphism

P69 Orai1α but not Orai1 β participates in constitutive Ca^{2+} entry and luminal breast cancer cell physiology

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Introduction: The proper operation of several cellular functions depends on the fine tuning of the cytosolic Ca^{2+} concentration, including those functions that are considered hallmarks of cancer cell physiology. The secretory pathway Ca^{2+}-ATPase, SPCA2 regulates the constitutive Ca^{2+} entry in luminal breast cancer cells and participates in the Golgi Ca^{2+} secretory pathway. The later mechanism would be responsible

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for Ca^{2+} transport to the milk during lactation and for the extracellular deposition of microcalcifications observed in aged females or in breast cancer patients. Such microcalcifications constitute a prognostic marker of mammary lesions. Reduced cytosolic Ca^{2+} concentrations in the absence of SPCA2 decrease the proliferation, migration, and tumorigenesis of cancer cells. It has been proposed that SPCA2 involves Orai1 in constitutive Ca^{2+} entry through a close intermolecular interaction that leads to constitutive Orai1 activation. Two Orai1 isoforms have been identified, the full - length Orai1α, consisting of 301 amino acids, the short variant Orai1β, lacking the 63 or 70 N-terminal amino acids comprising residues involved in channel inactivation and binding sites with Orai1 partners, We report here that only the mammalian-specific Orai1α participates in SPAC2-mediated constitutive Ca^{2+} entry in luminal breast cancer MCF7 cells.

Results: CRISPR engineered Orai1 KO MCF7 cells (KO) lack the constitutive Ca^{2+} entry observed in wild type (WT) MCF7 cells. Overexpression of Orai1α but not Orai1β is able to rescue constitutive Ca^{2+} entry in Orai1-knockout MCF7 cells. FRET analysis and immunoprecipitation revealed that Orai1α shows a greater ability to interact with SPAC2 than Orai1β. Deletion of the first 38 amino acids in Orai1α reduced the interaction with SPCA2 to a similar exent as Orai1β, thus suggesting that the N-terminal 38 amino acids play a relevant role in the Orai1β-SPCA2 interaction. Finally, we were able to induce extracellular *in vitro* microcalcifications in MCF7 cells overexpressing Orai1α, but not Orai1β, indicating that only Orai1α participates in the Golgi Ca^{2+} secretory pathway.

Conclusions: These findings support the functional role of Orai1α in constitutive Ca^{2+} entry and cell physiology in MCF7 cells, which might be a target for future therapies against breast cancer.

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Key words: Orai1α; Orai1β; constitutive Ca^{2+} entry; SPCA2; breast cancer; calcification

P70 Antidiabetic potential of FT50 in a mouse model of metabolic syndrome

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Introduction: Metabolic syndrome, the foremost metabolic disorder, is closely associated with the rising rates of obesity. It substantially increases the likelihood of cardiovascular disease and type 2 diabetes mellitus. Numerous global epidemiological studies consistently establish a correlation between high consumption of fats and carbohydrates and the onset of obesity. Similar changes have been observed in rodents, making them a valuable animal model for studying the pathophysiology of the metabolic syndrome.

Materials and methods: The objective of our study was to examine the impact of FT50, a plant extract, on the progression of metabolic syndrome and type 2 diabetes, focusing on its effects on obesity, glucose tolerance, and insulin resistance. Although the precise mechanism of action of FT50 remains incompletely understood, it is thought to inhibit the breakdown and absorption of carbohydrates in the gastrointestinal tract. These inhibitory properties are believed to contribute to the reduction of blood glucose levels, potentially providing therapeutic benefits for individuals with metabolic syndrome.

To assess this hypothesis, we exposed C57BL/6J male mice to a long-term (12-week) Western diet enriched with simple carbohydrates, a dietary pattern previously shown to induce metabolic syndrome. This dietary regimen resulted in the development of a complete metabolic syndrome in mice, characterized by significant weight gain, partially compensated type 2 diabetes mellitus with glucose intolerance and insulin resistance observed during intraperitoneal glucose and insulin tolerance tests.

Results: Incorporating FT50 in the Western diet effectively prevented the weight gain observed in the group fed solely the Western diet. Moreover, the addition of FT50 not only prevented the initiation of glucose intolerance and insulin resistance but also exhibited improvements in glucose tolerance and insulin sensitivity. The observed improvements in glucose tolerance and insulin sensitivity among mice receiving FT50 supplementation are of particular significance. This favorable outcome suggests a potential mechanism whereby FT50 mitigates metabolic dysregulation, possibly by interfering with glucose absorption in the gastrointestinal tract and insulin signaling in target tissues.

Conclusion: The findings from our study offer significant promise in advancing the development of plant extracts as potential antidiabetic treatments for human use.

Key words: metabolic syndrome, diabetes, plant extract, western diet, mouse model

P71 Effects of age-related chronic inflammation on bone mineral metabolism.

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Introduction/Objetives: Inflammaging, a state of low-grade chronic inflammation that occurs in elderly, is considered a risk factor for developing age-related diseases such as osteoporosis. Bone is a dynamic tissue, constantly maintained by a balance between formation, carried out by osteoblasts, and resorption, executed by osteoclasts. However, aging induces an imbalance in this process in favor of osteoclasts activity, with a consequent loss of bone density. Bone remodeling is also modulated by hormones such as parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and calcitriol, whose levels are altered in ageing. Here, we studied how chronic inflammation could affect bone mineral metabolism (BMM) and the molecular mechanisms involved.

Materials and methods: We first treated MLO-Y4 (mice osteocytes cell line) with serum of young wild type mice (2 months), old wild type mice (22 months) and mice with inflammation because of immunesenescence ($Tfam^{fl/fl}$ $Cd4^{Cre}$) alone or in combination with anti-IL-6 blocking antibody. Then, we analyzed gene expression of bone mineral metabolism related genes by real time PCR.

Results: Treatment of MLO-Y4 cells with old and immunosenescence mice serums induced an increase of mRNA expression of osteoclastogenesis and BMM related genes when compared with wild type young mice serum. The

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combination with anti-IL-6 blocking antibody did not show significantly changes in expression.
Conclusion: In this work we describe that chronic inflammation associated with aging promoted osteoclastogenesis regulation by osteocytes and this could be involved of impair bone regeneration during aging.
Key words: Inflammaging, bone resorption, osteocyte, inflammation

P72 Effects of exercise and diet on middle-aged rats fed with a high-fat diet

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Introduction/Objetives: Obesity, combined with high-fat diets and a sedentary lifestyle, poses a significant problem in today's developed societies. Evidence suggests that high-fat diets link with long-term health risks, including cognitive and motor impairments. This study aimed to determine the impact of a high-fat diet (HFD) on cognitive and motor function in middle-aged rats and to evaluate the protective effect of dietary changes and physical exercise.
Materials and methods: Female and male Wistar rats (24 of each sex, 5 months old) were fed a HFD for 3 months. After this period, one group continued the HFD while beginning a physical exercise program for 2 months. Two other groups also started the exercise program but switched to a standard pellet diet (HFD-SD) or an antioxidant-rich diet (HFD-Antiox). Additionally, one group continued on the HFD all the period (5 months), and another group received a standard diet for the full 5 months without participating in the exercise program (n=6 per group, each sex). Cognitive and motor functions were assessed using the Barnes maze and Rota-rod tests at both 3 and 5 months. Blood samples were taken at 5 months (upon sacrifice), and plasma levels of triglycerides and creatinine were analyzed.
Results: Rats of both sexes on the HFD regimen showed a decline in motor performance after 3 months, with no significant changes observed in the Barnes maze results. After 5 months, only the HFD-fed females exhibited cognitive decline in the Barnes maze, which was mitigated in females that participated in the exercise program and changed their diet. The 2-month physical exercise program led to improved motor performance in the Rota-rod test for both sexes. As expected, plasma triglyceride levels were higher in the HFD-fed animals of both sexes that were partially reverted with the interventions. Plasma creatinine levels were reduced in both sexes when HFD was followed, and a tendency to be recovered was observed in females after the diet change and exercise program
Conclusion: Physical exercise, combined with a healthy diet change, can counteract the negative effects of a high-fat diet on cognitive and motor abilities, with more pronounced benefits observed in females.
Key words: obesity, HFD, cognition, motor skills, physical exercis

P73 Grape Pomace flour prevents anxiety-like behavior and reduces brain oxidative stress in a type 2 Diabetes Mellitus animal model

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Introduction/Objetives: Type 2 Diabetes Mellitus (T2DM) is a complex metabolic disorder that heightens increases the risk of oxidative imbalance and cognitive impairments. Grape pomace flour (GPF), obtained after grape processing, has been evaluated as a potential therapeutic resource for T2DM treatment and prevention. This study examined the effects of GPF derived from 'Arinto' and 'Touriga Nacional' grape varieties on oxidative stress parameters in the cerebral cortex and hippocampus, as well as behavioral changes, in adult male Wistar rats.
Materials and methods: T2DM was induced by a high-fat diet (HFD) for 28 days, along with a single dose of streptozotocin (35 mg/kg) on the 21st day. Rats were divided into five groups: Control (CT), T2DM, T2DM+Metformin (250 mg/kg), T2DM+10% 'Arinto' GPF (WGPF), and T2DM+10% 'Touriga Nacional' GPF (RGPF).
Results: T2DM induction led to significant anxiety-like behavior. Oxidative stress evaluation revealed increased levels of nitrites and reduced catalase (CAT) activity in the cerebral cortex, with elevated reactive oxygen species (ROS) levels in the hippocampus. Treatment with both GPF and Metformin increased thiol content, reduced TBARS levels, increased CAT activity, and significantly reduced ROS levels with RGPF treatment in the cerebral cortex. In the hippocampus, both GPF treatments reduced SOD activity. Conversely, treatment with WGPF and RGPF demonstrated significant protective effects against anxiety-like behavior.
Conclusion: These findings suggest that grape pomace derivatives hold promise in mitigating the deleterious effects of T2DM in the brain, thus contributing to therapeutic and dietary interventions for health improvement.
Keywords: grape pomace flour; anxiety-like behavior; brain oxidative stress

P74 Diurnal Variation of Plasma Glucose Metabolism in the Postprandial Period in Normal Weight Women

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Introduction: Every living organism is exposed to the rhythmic influence of the environment, which is linked to the rotation of the Earth around the Sun, subjecting it to the organism's behavior and metabolism, which is regulated according to the 24-hour rhythmic pattern. On the other hand, circadian desynchronisation may be associated with several diseases, the mechanisms of which are still unclear. One of the reasons for the circadian desynchronization of metabolism is

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the difference in postprandial metabolism in the morning and evening when there is a difference in the secretion of metabolism-regulating signals and the sensitivity of their receptors.
he study aimed to determine the effects of a high-carbohydrate meal on the regulation of carbohydrate metabolism after a morning and evening meal in metabolically healthy young women with normal weight.
Materials and methods: A pilot study was conducted involving 7 women aged 20-23 with a body mass index of 20.0-21.9 kg/m2. Body fat mass and visceral fat level was determined by ultrasound method. To characterize postprandial carbohydrate metabolism, plasma glucose and insulin levels and salivary cortisol concentration were determined before the meal and twice during the postprandial period at 30 and 150 minutes after the meal. s a meal, the study participants took food with a calorie content of 10 kcal/kg of body mass with a nutrient ratio of 66% carbohydrates, 24% fat, and 10% protein. Meal and carbohydrate metabolism characteristics were determined between 7 am and 11 am for the morning visits and between 6 pm and 10 pm for the evening visits, on two different days. Participants' chronotype was determined using a validated questionnaire. The study was approved by the local ethics committee.
Results: The results of the study show that the fluctuations of morning postprandial plasma glucose are not statistically significant in the three-hour period after the meal, indicating an effective regulation of glucose metabolism in the morning hours, while in the evening, there is a significant increase in plasma glucose in the postprandial period, and it has a positive correlation (r = 0,4; p=0,04) with plasma insulin changes, but not with fluctuations in cortisol level.
Conclusion: It can be concluded that normal weight women have diurnal variations in postprandial glucose regulation effects, due to the timing of meals but is not linked to personal chronotype.
Key words: postprandial glucose, insulin, cortisol, woman

P75 Review and implementation of the technical criteria imposed by EFSA on the datasets included in national food composition databases (FCDB), within the framework of EuroFIR.

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Introduction: The contract NP/EFSA/IDATA/2022/01 was awarded by EFSA to EuroFIR AISBL. At this stage we have reviewed and implemented the technical criteria imposed by EFSA on the datasets included in national food composition databases (FCDB).
Methods:1. EuroFIR Technical Annex Fields. 2. Additional Fields Included in Generic FCDB.
Results: BEDCA and the rest of the FCDB participating in this project have carried out an in-depth and joint study of n=18 items (fields) and n=207 subitems (subfields), (n=207), in order to include said FCDB in open access in Europe, according to the quality guidelines set by EFSA.

1. Food Entity: a. Food Name and identifiers (5) b. Other food identifiers (4) c. Food classifications and descriptions (19) d. Labelling information (9) e. Other properties of foods (8)
2. Foodnames Entity (6)
3. Recipe Entity (9)
4. Ingredients Entity (7)
5. Retention Factors Entity (6)
6. Sample Entity (12)
7. Component Entity (5)
8. Method Specification Entity (6)
9. Analytical Key Steps (7)
10. Laboratory Performance (11)
11. Value Entity (12)
12. Analytical Statistics (3)
13. Other Statistics Results (6)
14. Method (5)
15. Quality Assesment Entity (4)
16. EuroFIR QA (12)
17. Contributing Values Entity (4)
18. Reference Entity (10). a. Book (4) b. Journal (Issue) (5) c. Journal Article (6) d. Report (3) e. Article in Report (6) f. File/Database (3) g. Software (2) h. Authoritative Document (3) i. Product Label (1)
19. Thirty fields included in Generic FCDB.

Conclusion: BEDCA meets the quality criteria required by EFSA
Key words: Data Quality, EuroFIR, EFSA, BEDCA, Food Composition Database.

P76 Relationship between the dietary intake of bisphenols in school children, the determination of obesogens in hair and its relationship with overweight/obesity

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Background and objectives: Endocrine disruptors (EDCs) may be related to exposure to obesogenic chemical contaminants, such as bisphenols in food packaging and some parabens in additives food.
Methods: In a previous study, 98 samples of foods selected from among the most consumed were analyzed, 56 of which had concentrations of parabens. The food frequency questionnaire (FFQ) was used to estimate dietary exposure to different parabens, focusing on foods that accounted for more than 95% of energy and nutrient intake. Logistic regression models were used to assess the influence of dietary exposure to parabens in overweight and/or obesity.
124 cases and 179 controls aged 6 and 12 years were recruited, from whom sociodemographic and lifestyle data. Dietary and

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anthropometric data as well as hair samples, were obtained. UHPLC-MS/MS was used to detect and quantify obesogens (4 bisphenols and 5 parabens) in hair and logistic regression models to evaluate their relationship with overweight and obesity.
Results and Conclusion: The results showed a positive association between MetPB, ButPB and total parabens present in meat and egg samples in children, and PropPB for meat and eggs in overweight and/or obese girls. A greater probability of overweight and obesity was observed in girls exposed to high levels of BPA from meat and eggs. Methylparaben (MetPB) and bisphenol A (BPA) were identified as the main compounds present in the hair samples. However, no statistically significant results were found on the association of bisphenols and parabens with childhood overweight and/or obesity.
Key words: Overweight; Pediatric obesity; Children; Bisphenols; Parabens.

P77 Role of exosome markers, FGF-Klotho-system and cfDNA as potential biomarker of premature-aging in adults with obesity.

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Introduction/Objetives: Premature aging is associated with obesity, elevated levels of exosomes and circulating free DNA (cfDNA). FGF-Klotho system plays a protective role in the pathophysiology of obesity due to its regulatory effects on chronic inflammation and elevated oxidative stress. This study aimed to assess the diagnostic potential of molecular biomarkers (FGF19, FGF21, FGF23, klotho, klotho beta, cfDNA, and plasma-derived exosome markers) in the development of obesity and premature aging in patients with obesity.
Materials and methods: Seventy-five men and postmenopausal women (aged 50-60) were divided into an Obesity Group (OG; n=50), with a body mass index ≥30 kg/m2, and a Control Group (CG; n=25), with healthy individuals without obesity. The expression of cfDNA, exosomal markers (CD9, CD63, CD81, and Flotillin-1), CD14 receptor, heat shock protein HSP90, and the protein expression of FGF19, FGF21, FGF23, Klotho, and Klotho-beta were analyzed by Western Blot.
Results: OG showed increased levels of cfDNA, exosomal CD81, CD63 and HSP90. A non-significant increase in CD14 was observed in the OG. Moreover, a significant decrease in Klotho protein expression was observed in the OG. No differences were found in the rest of exosomal and FGF-klotho system protein markers.
Conclusion: Results suggest the potential involvement of exosomes and the FGF-klotho system in the onset of obesity and premature aging. Further research is necessary to validate the significance of the FGF-Klotho system, along with exosomal markers and other biomarkers associated with inflammation and aging in individuals with obesity.
Key words: Obesity, premature-aging, FGF-Klotho system

P78 Complete Blood Count in a Rat Model of Metabolic Syndrome and Following Reduced Caloric Intake

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Objectives: Metabolic syndrome (MetS) is closely related to increase in oxidative stress and proinflammatory conditions. One of the first therapeutic approaches for MetS management is lifestyle modification, such as reduced caloric intake. We aimed to check whether the MetS induced by high-fat diet (HFD) modifies the complete blood count (CBC) parameters as well as to check the effect of fasting.
Materials and methods: We worked with following groups of male Wistar rats: control, HFD (feeding a 45% high-fat diet for 10 weeks) and HFD+fasting (HFD for 6 weeks; standard diet (20% reduction) for 2 weeks; 35% daily calorie intake reduction 2 weeks). CBC was analyzed by the use of Abacus Vet5 analyzer.
Results: We did not observed differences in the leucocyte count in general, however, monocyte count was greater in HFD while reduced after fasting. The percentage of lymphocytes were increased in HFD and not affected by fasting. Contrariwise, fasting resulted in decrease in the portion of monocytes and neutrophils. Erythrocyte count, hemoglobin concentration and hematocrit were lower in HFD and normalized after fasting. The platelet count was not modified by any intervention, but platelet distribution width was lower in HFD, while not affected by fasting.
Conclusion: In summary, while a CBC is not used to diagnose MetS directly, certain CBC parameters can provide valuable insights into the inflammatory and hematological changes associated with MetS. This can aid in early detection, monitoring, and risk assessment. Non-pharmacological intervention in a form of fasting showed normalization of several CBC parameters. Supported by: APVV-22-0154.
Key words: high-fat diet, fasting, complete blood count

P79 Comparison of Glucose-Stimulated Calcium Signaling in Human Pancreatic Tissues

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Introduction/Objectives: Understanding how pancreatic cells respond to glucose is vital for comprehending type 2 diabetes (T2D), and while animal models offer great insight, they don't fully capture the specific differences in human pancreatic function. This study aims to directly compare the glucose-stimulated calcium signaling and insulin secretion in human pancreatic tissue slices and isolated islets from non-diabetic and diabetic donors. seeking to better understand the cellular changes in the pancreas associated with diabetes.

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Materials and methods: Human pancreata were obtained from three deceased donors (two non-diabetic, one diabetic). Tissue slices were prepared from the tail region and glucose-stimulated calcium dynamics in response to increasing glucose levels (3, 6, 9, and 12 mM) were observed. Islets were isolated through enzymatic digestion for secretory function studies.
Results: Perifused tissue slices revealed varied calcium, with some cells active at 3 mM glucose and others responding to higher concentrations with fast oscillations. Non-diabetic islets showed a glucose-dependent increase in activity due to higher oscillation frequency, while diabetic islets lacked clear glucose dependence, despite a slight rise in activity between 3- and 12-mM glucose. Insulin secretion was significantly diminished in T2D donor islets compared to non-diabetic ones.
Conclusion: This study demonstrates fast glucose-dependent calcium oscillations corresponding with secretory responses in islets from the same donors. Non-diabetic islets exhibited enhanced glucose-dependent oscillation frequency, whereas diabetic islets showed reduced glucose responsiveness. These findings highlight a potential mechanism of beta-cell dysfunction in T2D, contributing to our understanding of the pathophysiological changes in human pancreatic islets in diabetes.
Key words: Beta cell, calcium imaging, Type 2 diabetes

P80 Pomegranate extract reverses cognition decline in aging frail mice and downregulates B2m gene expression in the cerebellum.

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Introduction/Objectives: Aging, the gradual deterioration of an organism, encompasses not only physical but also cognitive decline, including memory loss, anxiety, and reduced daily functioning. Recent studies implicate β2-microglobulin (B2m), a component of major histocompatibility complex class 1 (MHC I) molecules, in age-related cognitive and regenerative decline, suggesting it as a potential target for intervention in old age.
The aim of this work was to elucidate the effect of pomegranate extract (PE) during 4 months of supplementation in elderly mice to prevent the cognitive deterioration.
Materials and methods: The animal study was approved by the University of Valencia Ethics Committee for Research and Animal Welfare (License reference: A20201110122413). Male C57Bl/6J eighteen-month mice were assigned to one of two groups: control group or PE supplementation (150mg/kg/day) group. We evaluate, pre-supplementation and post-supplementation, burrowing test, Hole Ground Test and Elevated Plus Maze Test (EPMT), were conducted to assess daily activity, exploration, and anxiety-like behaviour, respectively. Additionally, B2m gene expression in brain tissues (cortex and cerebellum) was analyzed.
Results and conclusion: our results showed that 16 weeks of PE supplementation improved burrowing capacity and showed some positive effects on exploratory activity. However, no anxiolytic effects were observed in the EPMT. Furthermore, PE seemed to decrease B2m expression specifically in the cerebellum but not in the cortex. This suggests a potential link between PE supplementation, improved exploratory and burrowing skills, and reduced B2m expression in the cerebellum.
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Key words: aging, pomegranate extract, cognitive decline.

P81 Fructose induced liver and skeletal muscle dysfunction prevented by Bacillus clausii SF174 spores administration

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Introduction/Objetives: High dietary levels of fructose have a myriad of adverse effects. The main one is the ability to alter gut microbiota composition, inducing the development of "leaky gut" and the subsequent endotoxemia. This results in whole-body low-grade inflammation that can have a strong impact on two metabolically active tissues, the liver and the skeletal muscle. Currently, the use of probiotics as a therapeutical approach has grown exponentially. In particular, Bacillus clausii strains, thanks to its physiological ability to form spores, could ensure safe passage through the human gastrointestinal tract. In the light of the above consideration, we here evaluated the possible efficacy of B. Clausii SF174 in counteracting the deleterious effect induced by high fructose diet in above tissues.
Materials and methods: Male Wistar rats of 30 days were divided in 3 groups, fed a control diet (C), a fructose diet alone (F) or in combination with the daily administration 5×109 CFU of Bacillus clausii spores (sp174) for 6 weeks. Liver and skeletal muscle composition, insulin sensitivity, inflammation and oxidative balance were evaluated, with mitochondrial oxidative capacity.
Results: We here demonstrate for the first time the ability of Bacillus clausii SF174 to prevent the onset of low-grade inflammation and insulin resistance induced by fructose and preserving the mitochondria from alterations in the respiratory capacity in both liver and skeletal muscle, maintaining unaltered the oxidative balance. in counteracting the metabolic alterations induced in the liver and skeletal muscle by the high fructose diet.
Conclusion: Bacillus clausii SF174 spores administration is extremely efficient in counteract the fructose induced metabolic alterations, thus opening the way to the formulation of new preparations able to improve liver and skeletal muscle function, independently from the dietary habits.
Key words: liver, skeletal muscle, mitocondria

P82 Diurnal variation of postprandial metabolism in young women with different body mass index

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Introduction/Objectives: Meal timing has been associated with risks of developing obesity, but the mechanisms of such association remain unclear. Modern unlimited availability of food is also promoting increase in meal frequency and leads to

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more time spent in the postprandial period. The aim of this study was to assess energy expenditure, nutrients oxidation rate and salivary cortisol level in the postprandial period after breakfast and dinner in women with different body compositions.
Materials and methods: Twenty-one women participated in the study and were divided into three groups according to body mass index (BMI): underweight, normal weight, and overweight/obese. They were provided with a meal accounting for 10kcal/kg of body mass (66% carbohydrates, 24% fat, 10% protein). Indirect calorimetry measurements were performed during a three-hour period after the meal. Each participant attended two study visits - one in the morning and one in the evening.
Results: There was a significant difference (p=0,02) in energy expenditure between the overweight/obese and underweight groups during the 90-120 minutes postprandial period in the evening. Lipid oxidation rate increased after the meal for all groups in both visits, except for the overweight/obese group during the evening visit. No significant difference in cortisol level among BMI groups could be observed.
Conclusion: These results suggest that people with different BMI have different metabolic characteristics in different times of the day. Further studies with larger number of participants could elucidate the importance of meal timing and support nutrition and healthcare specialists in developing a more individualized approach for their patients.
Key words: Postprandial metabolism, energy expenditure, meal timing, salivary cortisol

P83 Effect of nicotinamide adenine dinucleotide mechanism on breast tumor cell lines

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Introduction: In treating cancer, alternative methods with fewer side effects and targeted treatment are increasingly being used, moving away from traditional methods. For this purpose, we aimed to provide nicotinamide adenine dinucleotide (NAD+) metabolism regulation associated with increased nicotine phosphoribosyl transferase (NAMPT) in cancer cells by targeting (1).
Material and Methods: In separate groups, two breast cancer cells, MCF7 and MDAMB231, were treated with 10 mM FK866, 40 mM quercetin, and 10 mM apigenin respectively. Various factors were measured and analyzed, including NAD/NADH rate, reactive oxygen species (ROS), glutathione-s-transferase (GST) activities, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α levels.
Results: Our data indicates that NAD/NADH levels differed statistically between MCF7 and MDAMB231 cells ($p<0.05$). The NAD/NADH ratio was low in both cell types with FK866, and high in both cell lines treated with apigenin. While ROS levels were low and GSTs levels were high in the groups using quercetin ($p<0.05$). The IL-1β and TNFα levels in MCF-7 cells were lower in the quercetin and FK866-treated groups than in the apigenin-treated group. However, in MDAMB231 cells, the levels were higher due to quercetin treatment.
Conclusions: Our findings suggest that although apigenin inhibits NAD+ metabolism, quercetin, with a stronger antioxidant effect, can support the biological production stage of cancer thanks to the high GST levels and low ROS levels of cancer cells. The researcher was supported in the scope of 2214-A - International Research Fellowship Programme for PhD Students by The Scientific and Technological Research Council of Turkey. Reference: 1.Wang B, Hasan M, Alvarado E, Yuan H, Wu H, Chen W. NAMPT overexpression in prostate cancer and its contribution to tumor cell survival and stress response. Oncogene. 2011;30(8):907-21.
Key words: MCF7, MDAMB231, FK866, quercetin, apigenin

P84 Role of EFCAB13 in SOCE activation

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Changes in Ca^{2+} homeostasis are crucial for the activation of intracellular mechanisms that lead to platelet aggregation. Deregulation of these mechanisms has been reported in several thrombotic pathologies like Stormorken or grey-platelet syndromes and, also in physiological events like birth. Neonatal platelets present an altered Ca^{2+}-release from internal stores, but also exhibits altered Ca^{2+}-entry through SOCE activation. In previous works, we have described altered expression of STIM1 and SARAF, and their effects in SOCE in neonatal platelets. We analysed in the present study another calcium-binding protein, EFCAB13, whose role in Ca^{2+} homeostasis is still unknown, despite changes in its expression has been reported in several pathologies like cancer or neurological diseases. Thus, reduced expression of EFCAB13 was evidenced in neonatal platelets respect their mothers in both at mRNA and protein levels. EFCAB13 coimmunoprecipitates with STIM1 and Orai1 during TG-evoked SOCE activation. Silencing of EFCAB13 almost completely impaired SOCE in MEG-01 cells. Colocalization of STIM1 and Orai1 was reduced by transfecting MEG-01 cells with the shEFCAB13 plasmid. Interestingly, EFCAB13 is capable of coimmunoprecipate with Orai1 in HEK293 STIM1 & 2 double KO cells, thus, revealing a direct regulatory role of this protein over Orai1 channel. Interestingly, EFCAB13 was randomly distributed within the cell under resting conditions, but it partially co-localized with the endoplasmic reticulum of MEG01 cells during TG-evoked SOCE activation. Finally, MEG-01 cells transfected with shEFCAB13 presented altered MEG01 maturation process. Overall, we conclude that EFCAB13 is a novel Orai1 activator that is crucial for Ca^{2+} homeostasis and, hence, its alteration may be underneath of some illnesses described in the platelet linage.
Key words: EFCAB13, STIM1, SOCE, platelets, MEG01

Environmental and exercise physiology

P85 Concurrent training in patients with hypertrophic cardiomyopathy: preliminary results.

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Introduction/Objetives: Physical exercise has emerged as a complementary treatment strategy for hypertrophic cardiomyopathy, and it is theorized to potentially improve functional capacity, exercise tolerance, and reverse adverse cardiac remodeling, among other benefits. Several variables derived from cardiopulmonary exercise tests (CPET), such as VO2max, VO_2 at the second ventilatory threshold (VT2), and ventilatory efficiency are associated with survival, making their improvement potentially impactful on prognosis. However, the practical implementation of physical exercise in patients with HCM is limited to 5 studies. A meta-analysis of their results evidenced an improvement in VO2max of 4.33 mL/kg/min and in NYHA functional class. The objective of this study was to assess the effect of a concurrent resistance and cardiorespiratory training program on CPET-derived and strength-related variables.
Materials and methods: Four HCM patients were evaluated before and after 12 weeks of individualized concurrent training with two sessions/week. Evaluations included body composition, CPET, echo- and electrocardiography. The first part of the sessions consisted of 6 resistance exercises, with 1-2 warm-up sets and 3 effective sets each, using a self-selected load that allowed them to execute 8-10 repetitions, leaving 6-8 in reserve. In the second part, one day per week was dedicated to continuous intensity, maintaining a heart rate 5-10 bpm above VT1; and one day per week was dedicated to fartlek training, alternating 2-6 min at a intensity 5-10 bpm below VT2 with 1-2 min of recovery walking. **Results:** Training promoted an average increase of 3,8 $ml \cdot kg^{-1} \cdot min^{-1}$ in VO2max (13% of baseline), 3 $ml \cdot kg^{-1} \cdot min^{-1}$ of VO_2 at VT2 (12% of baseline) and improvements of ventilatory efficiency at VT2 and VO2max intensities (-0,28 and - 0,38 for V'E/V'CO2, respectively), among other CPET-derived variables associated with a better prognosis and long- term survival. Muscular mass augmented as a result of training (1,25 kg on average), along with a mean increase of 51% in upper and lower body strength, while body fat decreased an 1,3% (0,77 kg on average).
Conclusion: The available data suggest a positive effect of concurrent training on patients' strength, functional capacity and CPET-derived variables that may improve their functional class, quality of life, and long-term survival. The replication of this protocol in a larger cohort of patients is warranted to confirm these preliminary results.
Key words: Exercise, prognosis, cardiac hypertrophy

P86 Effects of Social and Physical Enriched Environment on Sensorimotor and Social Performance in a Cerebellar Infarct Mice Model

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Introduction/Objetives: Cerebellar infarct (CI), a rare type of stroke can result in significant motor deficits such as ataxia and gait disorders, impacting daily activities and self-care, potentially leading individuals to experience social isolation. The present study aims to investigate effects of enriched environments as a therapeutic approach for CI in mice, assessing the influence of different environmental factors on the condition.
Materials and methods: Forty male C57BL/6 mice were divided into 5 groups: physical enrichment, enriched environment, enriched environment +3 healthy mice, standard housing, and sham. The CI model was induced in mice via stereotaxic injection of N5-(1-iminoethyl)-L-ornithine dihydrochloride. Sensorimotor and social performance were assessed pre-infarct and on specific days post-infarct using the wire walk test, footprint test, and three-chamber test. The infarct area was quantified by Hematoxylin & Eosin staining. Immunohistochemistry was employed to visualize Biorientation Defective 1 (BOD1), a factor associated with motor coordination, utilizing a confocal microscope. Data were analyzed by two-way ANOVA, with $p<0.05$ considered significant.
Results: CI in mice significantly impacted motor skills, balance, coordination, and social behavior, with subsequent modulation by environmental factors observed longitudinally ($p<0.01$). Notably, significant differences in step lengths among the groups were observed ($p<0.01$). Our analysis showed no significant divergence in step widths among the experimental groups.
Conclusion: These findings indicate that CI affects motor and social performance in mice negatively, emphasizing the pivotal role of environmental factors influencing deficits both positively and negatively. Our study provides crucial insights to innovative therapeutic and rehabilitation strategies.
Key words: Cerebellar Infarct, Enriched Environment, Motor & Social Behavior

P87 Comparison of heavy metal, total oxidant and antioxidant levels in umbilical cord blood of women living in rural and urban areas

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Introduction/Objectives: Long-term exposure to industrial waste can lead to the accumulation of heavy metals in humans through air inhalation, consumption of contaminated products, and water sources. The scope of this study was to compare the levels of total oxidant status (TOS), total antioxidant status (TAS), nickel (Ni), manganese (Mn), vanadium (V), selenium (Se), aluminium (Al), iron (Fe), mercury (Hg), and cadmium (Cd) in the umbilical blood of pregnant women in rural and urban districts.

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Materials and methods: Umbilical blood was collected from a total of 50 pregnant women residing in both rural and urban districts of Ankara, Turkey (n=25). To separate the plasma, the blood samples were centrifuged at 3000 rpm for 10 minutes. Total oxidant status, TAS by ELISA and heavy metals including Ni, Mn, V, Se, Al, Fe, Hg and Cd by ICP-OES were determined in the plasma. This study was supported by Kirikkale University Scientific Research Projects Unit within the scope of master's thesis project (Project No:2016/42).
Results: The study found that the levels of TOS, TAS, Ni, Mn, Al, Fe, Hg and Cd were significantly higher ($P<0.05$) in the umbilical blood samples of pregnant women in rural areas compared to those in urban districts, unlike V and Se.
Conclusion: This increase in oxidative and antioxidant activities in pregnant women living in rural areas with higher heavy metal exposure is considered to be exposure-induced oxidative stress and a defence mechanism against this stress, a system that protects both the mother and the offspring.
Key words: heavy metal, pregnancy, umbilical cord blood

P88 Decoding stress: the universal language of entropy production

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Introduction/Objectives: The accurate quantification of stress remains a formidable challenge in the realm of physiological research, largely owing to the intricate nature of the stress response and its potential overlap with other physiological processes. In this vein, our research introduces a novel metric: the stress entropic load (SEL), grounded in thermodynamic principles. SEL is mathematically construed as a time-dependent function, articulated through various heat and temperature alterations, as well as changes in respiratory gas exchanges. This measure is posited as a predictive tool for stress and metabolic alterations in homeothermic organisms, including humans.
Materials and methods: Our empirical exploration involved 12 healthy male volunteers, structured in a dual-phase experimental design. The initial phase focused on achieving a resting metabolic rate (RMR), guided by specific criteria encompassing oxygen uptake, carbon dioxide release, and respiratory quotient. In instances where RMR benchmarks were not met within a 40-minute window, the study progressed, presuming RMR attainment. The collected data from this segment were pivotal in calculating the body's entropy production at rest. The subsequent phase entailed participants engaging in tasks via the WinSCAT software, signifying the onset of the stress phase. Here, entropy production was scrutinized to gauge stress levels. Crucially, SEL was ascertained by contrasting entropy production during the RMR phase (serving as a baseline) against that recorded throughout the experiment's entirety.
Results/Discussion: Our findings indicate that SEL, as an indicator of specific entropy production, emerges as a viable marker of stress induced by prolonged mental effort. Notably, extended interaction with WinSCAT software markedly augmented SEL rates. The progression of SEL over time offered insights into when participants commenced stress-inducing tasks, underscoring its viability as a biological marker for mental exertion. Intriguingly, such stress did not manifest in conventional stress indicators like blood pressure or heart rate, though it did affect oxygen and carbon dioxide production. While these results lend credence to the utility of SEL as a stress marker, they are preliminary in nature, illuminating the prospective significance of SEL in the realms of stress measurement and physiological research.
Key words: stress, measurement, entropy, wearables

P89 Stress in advanced open water SCUBA divers

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Objective was to investigate changes in arterial blood pressure (ABP), heart rate (HR) and blood glucose concentration (BGC) in advanced SCUBA divers. Additionally, we used previously designed psychological tests, to measure level of negative automatic thoughts (NAT) and negative emotions (NE) in same divers.
Methods: 46 experienced recreational divers performed one dive to approximately 30 msw. ABP, HR and BGC were measured in each diver at baseline, immediately before and immediately after dive. Psychological testing was performed before and after diving.
Results: Divers on average: were 41.6 years old, had 14 years of experience and were diving 82 hours per year. Their body mass index was 25.2±3.8 kg/m^2. Diastolic AP increased from baseline (81.53±9.87 mmHg) to period immediately before the dive (87±15.51 mmHg), and stayed elevated after the dive (86.29±10.63 mmHg). Both HR and BGC decreased in period immediately after the dive (73.98±13.68 bpm, 5.45±1.22 mmol/L, respectively) comparing to baseline (83.18±14.39bpm, 6.2±1.36 mmol/L) and period before the dive (82.02±15.13 bpm, 6.02±1.84 mmol/L). Presence of NE, anxiety and stress all significantly decreased after a dive compared to baseline values. Divers that had three or more NAT had lover values of systolic, diastolic AP, HR and BGC compared with divers that had one or two NAT. However, decrease never reached level of statistical significance.
Conclusion: SCUBA diving changed cardiovascular and metabolic parameters observed in this study. Level of NE and stress decreased once stressful activity concluded. Understanding changes in AP, HR, BGC and stress during SCUBA diving can improve diver's safety.
Key words: cardiovascular changes, psychological testing, human diving

P90 Lactate accumulation is not a cause of the slow component of oxygen uptake kinetics

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Introduction: We tested the hypothesis that the increase in blood lactate concentration ([La]) above the maximal lactate steady state (MLSS) may cause the slow component (SC) of oxygen uptake (VO2) kinetics.
Methods: On fourteen males (age 25+6 years, mass 73+7 kg), we measured V'O2 and CO2 output (V'CO2) by metabolic cart (Quark CPET, Cosmed, Italy), heart rate (HR) by cardiotachography (Cosmed, Italy), [La], by an electro-

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enzymatic method (Arkray, Japan) on micro-blood samples from earlobe. Maximal V'O2 (V'O2max) on cycle ergometer was measured during incremental test. Maximal aerobic power (ẇmax) was obtained. Subsequent tests were at 60%, 65%, 70% and 80% of ẇmax, in random order. After 5 min at 50 W, the tested power was maintained until exhaustion. V'O2, V'CO2, and HR were monitored. [La] was determined at rest and every 5 min at exercise.
Results: V'O2max was 3.14+/-0.37 l/min. w'max was 279+31 W. At V'O2max, [La] was 11.0+/-2.9 mM, and gas exchange ratio (R) was 1.14+/-0.05. Maximal HR was 181+/-11 bpm. At 60 % ẇmax, all subjects attained steady state: VO2=2.35+/-0.21 l/min, V'CO2=2.22+/-0.22 l/min, R=0.94+/-0.03. HR was 158+/-14 bpm, [La] 4.5+/-2.4 mM. Power at MLSS was 175+/-22 W. At 80 % w'max, V'O2 and V'CO2 increased until the end of exercise. The mean V'O2 at min 10 was 3.03+/-0.26 l/min. Exercise lasted 11+/-3 min.
Conclusion: Energy balance computation after this data allowed refutation of the tested hypothesis. The relationship between [La] and size of SC denotes a correlation, not a mechanism. Type II fibres recruitment may explain this correlation.

P91 Fuel selection in recovery to dynamic exercise with blood flow restriction – a preliminary study

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Introduction/Objetives: In blood flow restriction (BFR) exercises, blood flow to the extremities is partially restricted by an inflated cuff to create a local hypoxic environment for the affected muscles during contraction. Acute changes in vascular, hormonal and metabolic responses to exercise have been reported during BFR exercise, but little attention has been paid to post-exercise recovery. Therefore, the aim of our study was to determine the metabolic effects of BFR post-exercise.
Materials and methods: After determining maximal oxygen consumption (VO2max), fifteen young recreational athletes (age 25.13±4.52, BMI 24.19±2.09) cycled at 40% and 60% VO2max for 5 minutes each, under control conditions (CTRL) and with both legs occluded at 80% of occlusion pressure. The trials were performed one week apart, the order was randomised. Oxygen consumption and carbon dioxide production were measured (QuarkPFT, Cosmed, Italy) to estimate the rates of carbohydrate (CHOox) and fat (FATox) oxidation before and 15 minutes in recovery according to Kelly L and Basset FA (2017). One-way ANOVA for repeated measures was performed to test for differences, followed by a post-hoc analysis with Bonferroni correction. A confidence level was $p < 0.05$.
Results: Fat oxidation was increased 15 minutes after exercise in BFR compared to CTRL (187±76 mg/min versus 148±49 mg/min, $p = 0.029$), while no difference was observed in carbohydrate oxidation rate (132±76 mg/min versus 171± 69 mg/min, $p = 0.58$).
Conclusion: BFR alters metabolism after exercise by promoting fat oxidation. Future studies should investigate the role of these findings in obesity and insulin resistance.
Key words: blood flow restriction, exercise, metabolism

P92 Cell polarity normalization as a component of anti-cancer effect by physical activity

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Introduction/Objectives: Ou MC decrescendo phenomenon exercise (OuDPe), a self-administered simple exercise, shows consistent anti-cancer effects. This study approached the mechanism of anti-cancer effect by physical activity via OuDPe.
Methods: The OuDPe is mainly performed by placing the contralateral hand directly on the body area via the left-right, dorsoventral or vertical axes to induce Ou MC decrescendo phenomenon (OuDP) and application along more axes has shown to be more effective.[1]
Results: Short-term(less than 6 months) OuDPe showed to suppress pancreatic (3/10), ovarian (1/10), and endometrial cancer growth (3/10), enhance chemotherapy effect of pancreatic cancer (1/10) and stop cancer-related bleeding (3/10) for 10 patients. Four patients received long term OuDPe (8 months to 6 years) with no concomitant anti-cancer treatment showed tumor regression but the advanced cancer of 3 patients (3/4) resumed growth after regression despite continuous OuDPe while the suspicious early pancreatic cancer of a patient (1/4) regressed completely and does not recur to date. OuDPe has showed a cause-effect anti-cancer effect. The pathology for the regression of endometrial cancer IIIB to IA by OuDPe showed apoptosis, restored apical-basal polarity, mitigated epithelial-mesenchymal transition, induced squamous metaplasia of cancer cells, which indicated cell polarity normalization of tumor cells.[2]
Conclusion: OuDPe has shown to normalize cell polarity and tissue function of cancer in the context of physical activity and human body anatomical axes, which underlines the most important anti-cancer mechanisms and may contribute to cancer prevention. It indicates normalization of cell polarity is associated with anti-cancer effects of physical activity.[1,3]
References: 1 Ou, MC., Ou, D., Pang, CC. FEPS, 2017, D15-1. 2 Ou, MC., Ou, D., Pang, CC., Ou, YJ. APJCP, 2023, 24, 2869-2874. 3 Ou MC, Chen FM. Ann Med Surg, 2024, 86:2137-2142.
Key words: cell polarity, physical activity, cancer

P93 Effect of ozone oxidative stress on potassium current in human lung cells: chemical mediators

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Introduction: Given that the ionic balance of the pulmonary epithelial lining fluid (ELF) is essential for correct gas exchange, our previous studies analyzed the effects of ozone, one of the most harmful pollutants for the respiratory system, on K+ currents in human cultured lung epithelial cells. O3 exposure (0.1 ppm, 30') significantly alters the flow of K+ ions, reducing the outward rectifier current component. O3 does not act directly, but through its bio-products, among which the main ones are 4HNE and H2O2.In this study we analyzed the action of 4HNE and H2O2 on K+ currents in second type pneumocytes and verified the protective effect of catalase.

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Material and Methods: A549 cellular line: lung epithelial cell model. 4HNE: 5, 10 and 20 uM concentration, 30'. H2O2: 20 uM concentration, 30'. Catalase: 1000U/ml concentration, 1.30h. Western blot and patch clamp technique: to study the action of the bio-products on K+ currents in A549 cells.
Results: 4HNE was able to significantly decrease the K+ current, but its action was less effective than O3. H2O2 also decreased the K+ current, but the effect did not reach the significance. Catalase shows the ability in preserving the outward rectifier component depressed by 4HNE, bringing back the current to the control level.
Conclusion: We can conclude that 4HNE is the main mediator of ozone, but its action also involves other compounds to a lesser extent. Further studies are needed to understand the pathway activated by 4HNE inside the cell and which pharmacological compounds can protect it.
Key words: pneumocytes, K^+ currents, O3 adducts.

P94 Maximal oxygen uptake relative to body mass is associated with mean and proximal skin temperaturas after maximal effort test

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Introduction/Objectives: This study aimed i) to determine the associations of VO_2 max relative to body mass (relative VO_2 max) with skin temperature (T_{sk}) parameters at the end of a maximal effort test, ii) to assess whether these associations differ between men and women, and iii) to investigate whether these associations are influenced by the participant's body mass index.
Materials and methods: 150 young adults (50 men and 100 women, aged 18–26) underwent a maximum effort test, with VO_2 max measured by indirect calorimetry. We monitored T_{sk} minute-by-minute using 13 iButtons during the test. Mean, proximal, and distal T_{sk} were calculated using standardised equations that combine measurements from different anatomical areas.
Results: In all participants relative VO_2 max was positively associated with mean T_{sk} and proximal T_{sk} at the end of the test (β=0.545, $p<0.001$; β=0.412, $p<0.001$). Similar associations were observed when the data were divided by gender. The associations between relative VO_2 max with mean T_{sk} and proximal T_{sk} were stronger in men than women (men all $R^2 \geq 0.487$; women all $R^2 \geq 0.138$). No significant associations were found between relative VO_2 max and distal T_{sk} or the supraclavicular T_{sk}. BMI did not influence the relationship between relative VO_2 max and mean/proximal T_{sk}, while time to exhaustion did.
Conclusion: Relative VO2 max was positively correlated with mean and proximal T_{sk}, with these associations being more pronounced in men. These relationships are independent of BMI. These results suggest that incorporating T_{sk} at the end of the test into current VO_2 max estimation models may improve accuracy for young, healthy adults.
Key words: thermoregulation, skin temperature, cardiometabolic health.

P95 Cell polarity normalization as a component of anti-cancer effect of physical activity – a clinical observation with Ou MC decrescendo phenomenon exercise

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Introduction: *Ou MC decrescendo phenomenon exercise (OuDPe), a self-administered simple exercise, shows consistent anti-cancer effects, which evinces the anti-cancer effect by physical activity [1, 2].*
Objective: To approach the mechanism of anti-cancer effect of physical activity.
Methods: The OuDPe is mainly performed by placing the contralateral hand directly on the body area via the left-right, dorsoventral or vertical axes to induce Ou MC decrescendo phenomenon (OuDP) and application along more axes has shown to be more effective.
Results: The OuDP has shown to produce a reproducible effect to reduce pain, inflammation, accelerate recovery of function of degenerative diseases and dysfunctional organ, and attenuate malignant changes. The resolution of non-infectious conditions with functional recovery of degenerative diseases and dysfunctional organ by OuDP indicates the restoration of normal tissue function [1]. OuDP has shown to suppress pancreatic, ovarian, and endometrial cancer growth, induce regression of early suspicious pancreatic cancer, enhance chemotherapy effect of pancreatic cancer and stop cancer-related bleeding [2]. In the regression of endometrial cancer, OuDP induced apoptosis, restored apical-basal polarity, mitigated epithelial-mesenchymal transition, induced squamous metaplasia of cancer cells, which were compatible with the phenomenon of cell polarity normalization [2]. Thus, the effect by OuDP is probably based on cell polarity normalization.
Conclusion: Spatial asymmetry, or establishment of cell polarity during embryogenesis by anteroposterior, dorsoventral, and left-right axes is crucial for normal tissue physiology and homeostasis. The tumor regression induced by OuDP demonstrated normalization of cell polarity of cancer cells [2]. It indicates normalization of cell polarity is associated with anti-cancer effects of physical activity.
Reference: 1. Ou, MC., Ou, D., Pang, CC., Ou, YJ. The Causal Relationship of Anti-Cancer Effect with Physical Activity Evinced by the Consistent Anti-Cancer Effect of the Ou MC Decrescendo Phenomenon. Asian Pacific Journal of Cancer Prevention, 2023, 24, 2869-2874. 2. Ou MC, Chen FM. The consistent anti-cancer effect of a simple exercise (Ou MC decrescendo phenomenon exercise) may hold promise for low-cost cancer prevention. Ann Med Surg (Lond), 2024, 86:2137-2142.

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P96 The intersection of artificial intelligence and natural physiological processes in the body - new trends in nutrition

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Introduction: Integrating artificial intelligence (AI) into the food sector revolutionizes how we approach diet and food production. Using AI, we can provide personalized dietary recommendations and optimize farming practices to meet the growing demand for sustainable food rich in essential micro- and macronutrients. Our research aims to explore how AI and natural physiological processes find a common path of development in nutrition to create innovative solutions for following a personalized therapeutic plan, sustainable food production and improved eating habits.
Materials and Methods: We comprehensively reviewed published scientific articles from 2018 to 2023 to gather the most up-to-date information on implementing AI in nutrition. Our search was primarily focused on reputable scientific web databases, including PubMed, Scopus, Web of Science, and Google Scholar, ensuring the reliability and accuracy of our findings.
Results: AI is used to create personalized nutrition programs, identify suitable functional foods, and monitor daily nutritional intake. Agriculture must also ensure precision yield and optimize supply to the end user. Among the trends in nutrition, there is a focus on intuitive eating, planned food intake, and smart appliances for storing and preparing meals.
Conclusions: This study highlights the significant trends and innovations at the intersection of AI and natural physiological processes in nutrition and illuminates their practical applications. The findings underscore the potential of technology to improve health outcomes and foster sustainable food systems, providing tangible benefits to individuals and the food industry.
Key words: artificial intelligence, nutrition, food intake

Epithelial transport

P97 Assessment of the Regenerative Potential of an Amphibian Skin Peptide on 3D Skin Models

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Background and Objectives: The skin has developed a complicated mechanism, the Wound Healing (WH) response, to close breaks in its barrier through growth factors. Chytridiomycosis, a pandemic disease brought on by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), is primarily to blame for the global decline in amphibian populations. Bd causes ulcers and significantly reduces an individual's fitness, though survival rates vary amongst species. The amphibian skin secretes antimicrobial peptides, involved in a variety of biological effects including accelerating the WH process and providing protection against UV radiation. This work aims to evaluate the skin healing potential of an amphibian peptide for future human application.
Material and Methods: Using bidimensional and three-dimensional model of WH, this work evaluates the WH effect of an amphibian skin peptide in three different forms: P1 (the complete peptide sequence), P2 (the first half of the sequence) and P3 (the second half). The WH activity was investigated on HaCat (Human keratinocytes cells) for 24h treatment using the inserts IBIDI Culture-insert 2 well. Once found the sample with the best WH activity, it was tested on EpiDerm 3D model (MatTek) for 72h treatment. Images of the wound were taken with a confocal microscope using DAPI and Falloidin, and the expression of four genes (MMP-1, MMP-9, FN-1, FLG) was obtained via qPCR.
Results and conclusions: The Results from HaCat test shows that P3 has higher WH activity than the other samples. This activity was confirmed on 3D models. The PCR data suggest tissues treated with the peptide express more genes involved in WH response respect to the negative control. These preliminary data are promising for a future application of this active principle for human use in the cosmetic or pharmaceutical fields. More data will be collected in the future to further confirm the remargination properties of amphibian skin peptides.
Key words: wound healing, 3D skin models, amphibian peptide.

P98 Positive impact of hydrolysed marine collagen using 3D skin models

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Background and Objectives: Collagen is the most abundant protein in the dermis of the human skin. This one is continuously deteriorated by different factors accelerating skin ageing. To this aim, the goal of this work is to evaluate the beneficial effect on 3D skin models treated with Hydrolysed marine collagen (HMC).
Material and Methods: HMC was characterized through hydroxyproline assay and UV spectroscopy. 3D skin models (MatTek) were used for this work; three different percentages (0.05%; 0.06%, 0.1% w/v) of HMC were solubilized in a phosphate-buffered saline (PBS) solution and also used as active ingredient within a cosmetic product. EpiDermFT were treated with 25 µL of each treatment topically; each tissues was then cultured for 24 hours. Then tissues were frozen or fixed for mRNA expression (AQP3, COL1A1, COL3A1, ELN) and histological analysis.
Results and conclusions: The presence of a 0.06% collagen solution leads to a higher expression of COL3A1 and AQP3 genes. Moreover, when examining COL1A1 gene expression, a notable increase is noted with the introduction of a 0.1% collagen solution. Additionally, a 0.05% collagen solution increases the expression of the ELN gene.
The cosmetic product with 0.1% collagen exhibits heightened expression levels of COL3A1 and COL1A1 genes. In contrast, cosmetic product with 0.05% HMC boosts the expression of

the ELN gene, while the cosmetic product incorporating 0.06% collagen demonstrates an upregulation of the AQP3 gene.
HMC is able to increase gene expression on 3D skin models, enhancing anti-wrinkle effect, especially when used within a cosmetic product.
Key words: Hydrolyzed marine collagen, 3D skin models, anti-aging effect.

Gastrointestinal and liver physiology

P99 Promising diagnosis of pancreatic cancer based on analysing the protein profile of circulating human-serum and plasma exosomes.

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Introduction/Objetives: Cancer death rate has descended in recent years; however, the lethality of some tumours still remains high. Pancreatic cancer is one of those. The aggressive tumour biology, late detection and inappropriate therapies contribute to the poor prognosis.
Extracellular vesicles (EVs) are a varied population of cell-derived membrane vesicles which convey proteins, nucleic acids, lipids and other metabolites. The cargo and surface-cell molecules determine the EVs functions and depend on the cell type. Exosomes are the smallest group of EVs (<150nm). Nowadays, it is possible to collect exosomes from human using non-invasive methods.
Materials and methods: Patients (n = 12) and healthy donors (n = 11) blood samples were provided from University Hospital of Cáceres. Serum and plasma were ultracentrifuged to collect exosomes. We employed imaging flow cytometry (ImageStreamX, Amnis) to confirm the quality of the extraction and the exosome presence. Their size distribution was examined by Dynamic Light Scattering (DLS) and compared with calibration beads. Exosomal protein profile was analysed by MS-based proteomics and differences were positive with a fold change ≥ ±2. The outcomes were validated by WB.
Results: We identified seven possible proteins to distinguish between healthy and pancreatic cancer patients. We focused on gelsolin which decreased -23.0-fold and serpin3 which increased +3,49-fold in patient samples compared to donors.
Conclusion: Our study showed two possible biomarkers for early detection of pancreatic cancer. Nevertheless, further research is needed to improve the diagnostic panel and to diminish the high mortality rates.
Funding: Cándido Ortiz-Placín was awarded a grant of Spanish Association Against Cancer (AECC).
Key words: exosomes; pancreatic cancer diagnosis; proteomics.

P100 Sigma 1 receptors as modulators of visceral pain: Lessons from knock-out rats

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Introduction/Objectives: Evidence suggests that Sigma 1 receptors (σ1R) play a modulatory role in somatic and visceral pain, eliciting analgesic actions. To further characterize the potential role of σ1R on visceral pain we used σ1R knock-out rats to assess changes in visceral pain in a model of colorrectal distension (CRD) in conscious animals.
Materials & methods: Wild-type (WT, n=5) and σ1R knock-out (KO, n=9) adult female rats were used. CRD (phasic-ascending distensions, 10-80 mmHg; repetitive noxious distension, 80 mmHg x 30 s x 12 distension) was performed using a barostat. Contractions of the abdominal musculature (visceromotor response) served as a surrogate marker for the assessment of visceral pain. All procedures were approved by the ethical committee of the UAB (protocols 3958 and 3961).
Results: Basal visceromotor response was similar in both groups of animals. During a phasic-ascending CRD (10-80 mmHg), σ1R KO rats showed increased visceromotor responses to CRD (AUC; WT: 1.53±0.19; σ1R KO: 2.50±0.22; P<0.05), although the threshold for pain was similar in both groups (WT: 31.00±6.40 mmHg; σ1R KO: 27.78±3.19 mmHg; P>0.05). During a repetitive noxious CRD (80 mmHg) visceromotor responses in σ1R KO rats were enhanced (AUC; 4.03±0.34; P<0.05 vs. WT: 2.51±0.27). Morphine (0.02-10 mg/kg, sc) Produced a similar dose-related inhibition of the visceromotor responses to noxious CRD (80 mmHg), regardless the genotype considered.
Conclusions: Enhanced visceral pain responses to CRD in σ1R KO rats suggest that σ1R might play a physiological role in the modulation of visceral pain arising from the gastrointestinal tract, eliciting analgesic-like actions.
Key words: Gastrointestinal; Pain; Sigma receptors; Visceral pain.

P101 Postbiotics of *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*attenuates store-operated Ca2+entry and FAK phosphorylationin Caco-2 cells

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Ca^{2+} is a ubiquitous second messenger that participates in a variety of biochemical processes, regulating them. Therefore, the cytosolic concentration of Ca^{2+} is highly modulated by numerous mechanisms, among which store-operated Ca^{2+} entry (SOCE) stands out. This process involves the entry of extracellular Ca2+ through Orai channels in response to a decrease in Ca^{2+} concentration in intracellular stores, detected by STIM proteins. Colorectal adenocarcinoma cells exhibit a remodelling in the expression of proteins involved in SOCE, causing various molecular alterations (*Sobradillo, D. et al., 2014*). Additionally, it has been confirmed that the use of postbiotics has a beneficial effect by modifying the tumour microenvironment (*Vrzáčková, N. et al., 2021*).
In the present study, we analysed Ca^{2+} mobilization using the single-cell imaging technique in colorectal adenocarcinoma Caco-2 cells exposed to the postbiotic agents of *Lacticaseibacillus paracasei* CECT 9610 and *Lactiplantibacillus plantarum* CECT 9608, and we confirmed that they exhibit a lower Ca^{2+} entry compared to control cells. Subsequently, we corroborated that this decrease is due to

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lower expression of the proteins Orai1 and STIM1, two of the main regulators of SOCE. Finally, through the wound healing assay, it was confirmed that cells exposed to postbiotic agents have a reduced migration capacity and that the phosphorylation of the FAK protein, which is critical for this process, is also lower.
Considering the results obtained, we conclude that the expression of proteins involved in SOCE is altered in cells exposed to postbiotic agents, affecting intracellular Ca^{2+} mobilization and cell migration.
Sobradillo, D. *et al.* (2014) J. Biol. Chem. 289(42), 28765–28782. Vrzáčková, N. *et al.* (2021) Molecules 26(6), 1528.
Supported by PID2022-136279NB-C21 (MCIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe") and Junta de Extremadura-FEDER (IB20007 and GR18061).
Key words: colorectal cancer, postbiotics, Orai1

P102 Effects of long-term NRF2 activation by dimethyl fumarate on liver function under normal and stress conditions in borderline hypertensive rats

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Introduction/Objetives: We investigated the effects of long-term crowding stress, dimethyl fumarate (DMF) treatment and their interaction in the liver of borderline hypertensive rats. We investigated the expression of transcription nuclear factor (erythroid-derived 2)-like 2 (NRF2, encoded by *Nfe2l2*) and selected NFR2-regulated genes involved in antioxidant defence, inflammation and iron metabolism.
Materials and methods: Rats were exposed to crowding stress, DMF treatment (30 mg/kg/day), or a combination of both for six weeks. Plasma parameters were analysed using a biochemistry analyser and ELISA kit, gene expressions were analysed using RT-qPCR. Nitric oxide (NO) formation was measured by conversion of 3H-L-arginine to 3H-L-citrulline and oxidative damage by determination of conjugated diene levels.
Results: DMF treatment suppressed the *Nfe2l2* expression, which did not alter the expression of NRF2-regulated genes involved in antioxidant defence, inflammation, or iron metabolism, and did not lead to increased oxidative damage. NO production was increased by DMF but unaltered by stress. Stress upregulated expression of genes involved in inflammation (*Tnf*), iron (*Fpn1, Tfr1, Irp1, Dmt1, Fth1*) and lipids (*Ppara, Pparg*) metabolisms, but it reduced oxidative damage. Regarding plasma parameters, stress increased ALT, total cholesterol and corticosterone levels, while DMF reduced the ALP and increased corticosterone levels.
Conclusion: Our study showed that long-term NRF2 activation by DMF reduced *Nfe2l2* expression without altering the expressions of NRF2-regulated genes. Stress increased the expression of pro-inflammatory markers and affected iron and lipid metabolism but these alterations were not altered by co-treatment with DMF. The study was supported by the grants VEGA 2/0157/21 and APVV-22-0296.
Key words: NRF2, iron metabolism, oxidative stress

P103 Alamandine attenuates oxidative stress, inflammation and intestinal morphology on rats exposed to chronic unpredictable mild stress

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Introduction/Objetives: Chronic unpredictable mild stress model of depression characterized by a series of pathological changes. Renin-angiotensin system (RAS) also plays a role in the pathophysiology of major depression. Alamandine is the newest component associated with the protective axis of the RAS. Therefore, we aimed to investigate the effects of alamandine on gastrointestinal morphology, and systemic inflammation.
Materials and methods: Male Sprague Dawley rats divided into 5 groups of control (C), depression (D), alamandine (50 µg/kg, ip, 21 days) treatment group (D+A), A779 (300 µg/kg, ip, 21 days) treatment group (D+A779), both alamandine and A779 treatment group (D+ALA+A779, 21 days). Depression groups subjected to chronic unpredictable stress protocol for 35 days. At the end of the experiment, blood samples, jejunum and colon tissue samples were obtained.
Results: Levels of serum AST and ALT were significantly higher in D and D+A779 groups than the D+ALA and D+A+A groups. Serum inflammatory markers such as IL-1β and IL-6 were significantly lower in D+ALA and D+A+A groups than the D group and the antioxidant marker, SOD levels were significantly higher in both D+ALA and D+A+A groups than the D group. The villi and epithelium degeneration and inflammatory cell infiltration in the lamina propria were detected in D, D+Ala and D+ A779 groups, while D+A+A group had more regular villus structure, as the control group. There were not any morphological changes detected in the colon tissue.
Conclusion: The present data indicated that alamandine attenuates oxidative stress, systemic inflammation and intestinal morphology impaired by chronic unpredictable mild stress exposure.
Key words: Alamandine; Chronic Stress; Inflammation.

P104 Investigation of repeated aluminum exposure on the structural and functional integrity of a 3D intestinal tissue model

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Background and Objectives: One of the most prevalent elements in the crust of the Earth is aluminium (Al3+). The free cation of Al3+ is very physiologically reactive and might disrupt metabolism, perhaps causing gastrointestinal problems and neurotoxicity, which would raise the prevalence of Alzheimer's disease. Since soil contains a significant quantity of Al3+, the amount that plants absorb depends on how much of it is present there. Humans, indeed, consume Al3+ every day through their food and drink.
Material and methods: This study aimed to assess the impact of repeated exposure to varying concentrations of Al3+ on a 3D intestinal tissue model. Over a 12-day period, different Al3+ concentrations (5, 20, and 50 ppm) were applied on epiIntestinal 3D models (MatTek). The study employed multiple methods to evaluate the effects, including TEER measurements to assess tissue viability, ICP-AES analysis to quantify Al3+ trespass, histological assessment for tissue architecture, evaluation of tissue barrier integrity, transmission electron microscopy (TEM) analysis of microvilli structure, and mRNA expression analysis (MT1E, OCLN, CLDN) to investigate oxidative processes at the end of the 12-day exposure period.
Results: Toxicity was not observed across all tested Al3+ concentrations, as indicated by TEER measurements that showed no significant differences between the treatments and the negative control. These findings were further corroborated through histological examination of the tissues.
Discussion and Conclusion:
Overall, the results of this study suggest that the repeated exposure to Al3+ in the tested concentrations could lead a detrimental effect at the higher concentration.
Key word: Aluminum,3D tissue model, physiological exposure.

P105 Utility of gelsolin and serpin-3 conveyed by circulating exosomes as promising biomarkers for diagnosis of pancreatic cancer.

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Introduction/Objetives: Cancer death rate has descended in recent years; however, the lethality of some tumours still remains high. Pancreatic cancer is one of those. The aggressive tumour biology, late detection and inappropriate therapies contribute to the poor prognosis.
Extracellular vesicles (EVs) are a varied population of cell-derived membrane vesicles which convey proteins, nucleic acids, lipids and other metabolites. The cargo and surface-cell molecules determine the EVs functions and depend on the cell type. Exosomes are the smallest group of EVs (<150nm). Nowadays, it is possible to collect exosomes from human using non-invasive methods.
Materials and methods: Patients (n = 12) and healthy donors (n = 11) blood samples were provided from University Hospital of Cáceres. Serum and plasma were ultracentrifuged to collect exosomes. We employed imaging flow cytometry (ImageStreamX, Amnis) to confirm the quality of the extraction and the exosome presence. Their size distribution was examined by Dynamic Light Scattering (DLS) and compared with calibration beads. Exosomal protein profile was analysed by MS-based proteomics and differences were positive with a fold change ≥ ±2. The outcomes were validated by WB.
Results: We identified seven possible proteins to distinguish between healthy and pancreatic cancer patients. We focused on gelsolin which decreased -23.0-fold and serpin3 which increased +3,49-fold in patient samples compared to donors.
Conclusion: Our study showed two possible biomarkers for early detection of pancreatic cancer. Nevertheless, further research is needed to improve the diagnostic panel and to diminish the high mortality rates.
Funding: Cándido Ortiz-Placín was awarded a grant of Spanish Association Against Cancer (AECC).
Key words: exosomes; pancreatic cancer diagnosis; proteomics.

P106 Phoenixin-14 alleviates inflammation, oxidative stress, and apoptosis in a rat model of acetic acid-induced ulcerative colitis

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Introduction/Objectives: The anti-inflammatory and antioxidant effects of phoenixin (PNX)-14 have been shown in different disease models. We aimed to investigate the effects of PNX-14 in acetic acid (AA)-induced ulcerative colitis.
Materials and methods: Rats were administered 1ml saline (control group) or 5%AA (colitis groups) intrarectally. Control group was treated with saline intraperitoneally; colitis groups were treated intraperitoneally either with saline or PNX-14 (50µg/kg/d) or cetrorelix (CTX; 100µg/kg/d) or CTX before PNX-14 or sulfasalazine (100mg/kg/d; positive control) immediately after and once daily for 3 days following the colitis induction. Colonic samples were taken for the histological and biochemical assessments [myeloperoxidase, malondialdehyde, glutathione, chemiluminescence, pro-inflammatory cytokines, caspase-3, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) measurements] on the 3rd day. This study was approved by the University of Health Sciences Animal Care and Use Committee (2022-04/03), supported by University of Health Sciences Research Fund (2022/201) and TUBITAK (122S979).
Results: High macroscopic and microscopic damage scores, myeloperoxidase, malondialdehyde, caspase-3, cytokine, and chemiluminescence values, and a decrease in glutathione levels of the colitis group were reversed by PNX-14 treatment ($p<0.05$-0.001). CTX or CTX before PNX-14 treatment diminished damage scores, caspase-3, 8-OHdG, cytokine, and chemiluminescence values ($p<0.05$-0.001). Sulfasalazine treatment improved all measurements except malondialdehyde and glutathione.
Conclusion: PNX-14, which ameliorates macroscopic, histological and biochemical parameters, can be considered as a potential therapeutic agent in ulcerative colitis with its anti-inflammatory, antioxidant and anti-apoptotic effects.

Additionally, it was demonstrated for the first time that CTX, which antagonizes the effects of PNX-14 in different studies, has similar effects with PNX-14 in this model.
Key words: ulcerative colitis, phoenixin, oxidative injury

P107 Gilaburu (Viburnum opulus L.) fruit extract ameliorates burn-induced distant organ injury via anti-inflammatory, antioxidant, and anti-apoptotic mechanisms in rats

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Introduction/Objectives: Besides to local tissue damage, burn has damaging effects on distant organs and presents a clinical picture leading to multiple organ failure. Gilaburu (*Viburnum opulus* L.) is a fruit with rich polyphenol content and used ethnobotanically in Turkiye. This study aimed to investigate the effects of gilaburu fruit extract on burn-induced distant organ injury.
Materials and methods: Under anesthesia, dorsum of the rats was exposed to 90oC (burn) or 25oC (control) water-bath for 10s to induce partial-thickness-second-degree skin burn injury (BI) and treated orally either with saline (1 ml) or gilaburu (100 mg/kg/d) immediately and at 24h after the BI. In the pretreatment group, gilaburu administration was started 1 week before the BI. Rats were decapitated 48h after BI, histological and biochemical evaluations were performed. This study was approved by the Marmara University Animal Care and Use Committee (29.2018.mar).
Results: High liver enzymes in the serum, increased microscopic damage scores, myeloperoxidase, malondialdehyde, chemiluminescence values, and reduced glutathione levels in liver and intestinal tissues of the burn group were all reversed by gilaburu treatments ($p<0.05$-0.001). Increased smad-3 and interleukin (IL)-6 levels in intestinal and elevated caspase-3 and IL-8 levels in liver tissues were decreased by GB treatments ($p<0.05$-0.001). Elevated matrix metalloproteinase-9 level in liver tissue was not changed by GB treatments. 8-hydroxy-2'-deoxyguanosine, transforming growth factor-beta1, tumor necrosis factor-alpha, IL-1beta and interferon-gamma levels were not changed by BI in both tissues.
Conclusion: Gilaburu shows therapeutic and/or prophylactic effects against BI in distant organs by suppressing burn-induced inflammatory response, oxidative stress and apoptosis.
Key words: burn injury, *viburnum opulus* L., oxidative damage

P108 The effect of gold and silver nanoparticles capped with *Cornus mas* L. extract on PI3K-AKT signaling pathway genes expression in oral dysplastic keratinocytes

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Introduction/Objectives: The study aims to investigate the ability of gold (AuCM) and silver nanoparticles (AgCM) capped with *Cornus mas* L. (CM) extract to influence the gene expression involved in PI3K-AKT pathway in human dysplastic oral mucosa cells (DOK).
Materials and methods: DOK cultures were treated with AuCM, AgCM and CM extract. Untreated cells were used as controls. Viability, cell death by FACS (apoptosis/necrosis) and the gene expression by using RT2 PCR Profiler high-throughput technique were evaluated. Thus, the 96-well PCR plates containing RT2 qPCR Primer Assays for a set of 84 genes, five housekeeping genes, and three controls were assayed on qPCR (Roche LC480).
Results: In dysplastic cells, AgCM and AuCM significantly decreased cell viability and induced cell death at doses higher than 20 µg/ml compared to CM. Forty-seven genes with different expression levels were found, 27 genes being over-expressed while 20 genes were under-expressed. The results were integrated with other two signaling pathways (p53 and WNT) with potential therapeutic approach in cancer chemotherapy. Higher values of gene over-expression were identified for *MTOR*, *GSK3B*, *EIF4B*, and *RAC1* genes. The *mTOR* was under-expressed while *GSK3B* gene was over-expressed. Among the under-expressed genes, we outline those with more than 2-fold activity suppression respectively *EIF4E*, *RHOA*, and *RPS6KA1*.
Conclusions: In dysplastic cells, AgCM and AuCM significantly decreased cell viability and induced cell death at doses higher than 20 µg/ml. The suppression of *GSK-3B* activity under therapeutic interventions suggested that gold and silver nanoparticles treatments can be associated with inhibition of cancer progression.
Key words: oral cancer, metallic nanoparticles, genes

P109 Zileuton, an inhibitor of arachidonic acid metabolization through 5-lipoxygenase, modulates antioxidant response in rat pancreatic stellate cells.

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Introduction/Objetives: Arachidonic acid (AA) is a cellular membrane-constituent lipid that is thought to be involved in several pancreatic disorders, including pancreatitis, pancreatic cancer or diabetes. It can be metabolized by three different enzymes: the cyclooxygenase, the lipoxygenase, or the cytochrome P450. Here we analyzed the effect of the inhibition of 5-lipoxygenase (5-LOX) by zileuton on the physiology of rat pancreatic stellate cells (PSCs), a cell type that has been signaled as the major responsible for the development of pancreatic fibrosis.
Methods: Cell viability, the generation of reactive oxygen species (ROS), oxidized and reduced glutathione, the levels of TBARS and carbonyls and the expression of superoxide dismutase 1 and 2 as well as the phosphorylation of the nuclear factor erythroid 2-related factor (Nrf2) were studied.
Results: In the presence of zileuton cell viability was increased. Increases in TBARS and carbonyl levels were

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observed. No significant changes were observed in the GSH/GSSG ratio. Additionally, no increase in ROS production was detected. Finally, increases in the phosphorylation of Nrf2 and in the expression of SOD-1 and SOD-2 were noted.
Conclusion: Metabolization of AA through 5-LOX might influence red/ox state of PSCs. Because the oxidative state determines PSCs´ proliferation, further studies will be needed to unravel the role of 5-LOX in PSCs physiology and its involvement in the development of pancreatic fibrosis.
Funding: Study carried out under the project GR21037 (Junta de Extremadura-FEDER). Cándido Ortiz-Placín was awarded a grant of Spanish Association Against Cancer (AECC).
Key words: antioxidant, pancreatic stellate cells, zileuton.

P110 Bioinformatic identification of differentially expressed genes in hepatocarcinoma and their correlation with *HIF1A*

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Introduction/Objetives: In hepatocarcinoma (HCC), the third cause of cancer-related deaths, hypoxia is a key feature. Hypoxia-inducible factor 1α (HIF-1α) controls the expression of genes involved in angiogenesis, metabolism, cell survival, invasion and metastasis. We aimed to identify differentially expressed genes (DEGs) in human HCC samples and associated tumor processes, and their possible correlation with *HIF1A* and patients' survival.
Materials and methods: We performed a dataset screening of gene expression profiles in tumor *vs*. non-tumor tissue samples from HCC patients obtained from the NCBI GEO database. Datasets that met the eligibility criteria were selected for DEGs identification using GEO2R tool. Cancer-related processes genes were obtained from the GeneCards database for the functional identification of HCC DEGs through a Venn diagram. Moreover, association with *HIF1A* and overall survival (OS) were assessed employing TNMplot and Kaplan-Meier plotter, respectively.
Results: Three gene expression profiles were selected. Results exhibited that 37, 69, 44, 21, 10, 160 and 38 DEGs in HCC samples participated in apoptosis, proliferation, invasion, metastasis, angiogenesis, energy metabolism and immune evasion, respectively. Correlation with *HIF1A* was confirmed especially in DEGs involved in the last three processes mentioned. Kaplan-Meier OS analysis demonstrated that overexpression of 10 DEGs more associated with *HIF1A* was related with HCC prognosis, according to their positive or negative correlation with *HIF1A*.
Conclusion: Functional relevance of HIF-1α in angiogenesis, energy metabolism or immune evasion has been demonstrated given the correlation with DEGs in tumor and non-tumor samples from HCC patients. These findings could be useful to design new therapeutic strategies.
Key words: gene expression, hepatocarcinoma, hypoxia.

P111 Studying hypoxia through hypoxia-inducible factor 1α-mediated cell response in bi- and tri-dimensional hepatocarcinoma models

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Introduction/Objetives: Hepatocarcinoma (HCC) represents one of the deadliest types of cancer, being hypoxia, through the hypoxia-inducible factor 1α (HIF-1α), a key factor responsible for its high incidence and therapeutic failure. This condition triggers the activation of several cancer-related molecular pathways, thus leading to poor patients' prognosis. Our objective was to evaluate the effect of HIF-1α on our HCC *in vitro* models.
Materials and methods: Analyses were performed in PLC/PRF/5 cell line employing $CoCl_2$ 100 µM to mimic hypoxia, being protein expression determined by Western blot. Two *HIF1A* knockout (KO) clones were obtained using CRISPR/Cas9 and spheroid-based tri-dimensional (3D) models were generated from both wild type and KO cell lines. Tumor growth was monitored, and hypoxia was assessed using the Image-iT Red Hypoxia Reagent, while cell death was determined by calcein-AM with propidium iodide staining. Statistical analyses were conducted using GraphPad Prism 8, with significance set at $p < 0.05$.
Results: Under induced hypoxia, the PLC/PRF/5 cell line displayed elevated expression of HIF-1α. The *HIF1A* KO 3D models demonstrated slower growth rates compared to the wild type, with all HCC spheroids exhibiting a physiological hypoxia in the tumor core. Moreover, cell death in KO spheroids was notably increased, suggesting a crucial pro-survival role of HIF-1α in HCC.
Conclusion: HIF-1α expression is responsible for tumor cell survival under hypoxic conditions using 3D HCC models. This emphasizes the potential of inhibiting HIF-1α activity as a therapeutic target to manage tumor progression and enhance patients' survival.
Key words: 3D models, hepatocarcinoma, hypoxia.

P112 The Impact of Various Extracts of *Angelica purpurascens* (Avé-Lall.) Gilli on Caco-2 Cell Line

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Introduction/Objetives: The Caco-2 cell line is commonly utilized for *in-vitro* studies of prevalent type of cancer, human colorectal adenocarcinoma. *Angelica purpurascens* (AP) is a

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plant traditionally used to enhance the immune, circulatory, respiratory, and nervous systems and to treat tumors. This study aims to examine the cell viability, oxidant, and antioxidant effects of hexane and methanol extracts from the aerial parts of AP on the Caco-2 cell line.
Methods: Caco-2 cells were cultured in a suitable medium until they reached full confluence. Various concentrations of hexane and methanol AP extracts (3.90-500 µg/mL) were added to the medium and incubated for 72 hours. The cytotoxic effect on cell viability was assessed using the MTT assay. Total oxidant and antioxidant activities were measured using commercial kits.
Results: The methanol extract of AP exhibited an anti-proliferative effect on the Caco-2 cell line within the dose range of 3.90-500 µg/mL ($p<0.05$). The hexane extract showed anti-proliferative effects at concentrations between 62.50-500 µg/mL ($p<0.05$). Regarding antioxidant activity, both the methanol (15.60-500 µg/mL) and hexane (3.90-15.60 and 62.50-500 µg/mL) extracts demonstrated increased antioxidant status ($p>0.05$). For oxidant activity, the methanol extract showed a decrease at 31.25, 62.50, 250, and 500 µg/mL ($p<0.05$), while the hexane extract displayed a significant reduction in oxidant levels at 15.60, 250, and 500 µg/mL ($p<0.05$).
Conclusion: The study found that different extracts from the aerial parts of AP have dose-dependent anti-proliferative effects on the Caco-2 cell line. Further detailed studies are suggested to elucidate the pathways through which these anti-proliferative effects occur.
Key words: Human colorectal adenocarcinoma cancer cells, *Angelica purpurascens*, Caco-2 cell line, cell viability.

P113 The Impact of Different Part Extracts of *Peganum harmala* L. on Human Colorectal Adenocarcinoma Cell Line (Caco-2)

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Introduction/Objetives: The Caco-2 cell line, derived from colorectal cancer, is widely used as an intestinal model to evaluate drug membrane permeability. *Peganum harmala* (PH) is a plant known for its various biological activities. This study aims to evaluate the effects of methanol extracts from aerial parts and fruits of PH on the Caco-2 cell line, focusing on cell viability and oxidant-antioxidant status.
Methods: Caco-2 cells were cultured in an appropriate medium until they reached full confluence. Methanol extracts from the aerial parts and fruits of PH were tested in this study. Different concentrations of the PH extracts (3.90-500 µg/mL) were added to the medium and incubated for 72 hours. The cytotoxic effect on cell viability was assessed using the MTT assay. Total antioxidant and oxidant statuses were measured using commercial kits.
Results: The methanol extract of PH aerial parts showed an antiproliferative effect at concentrations ranging from 62.50 to 500 µg/mL ($p<0.05$). The methanol extract of PH fruits exhibited an antiproliferative effect at 500 µg/mL ($p<0.05$). An increase in total antioxidant status was observed in the 62.50-500 µg/mL dose range of PH aerial part extract compared to the control group ($p>0.05$). The total oxidant status decreased in the 125-500 µg/mL and 7.8-31.25 µg/mL dose ranges for PH aerial part and fruit extracts, respectively ($p>0.05$).
Conclusions: This study demonstrated that PH extracts have an antiproliferative effect on the Caco-2 cell line within a narrow therapeutic range. The extracts also influenced the antioxidant and oxidant status, although these effects were not significant.
Key words: *Peganum harmala*, Caco-2 cell line, cell viability.

P114 Gut microbiota alters the metabolomic status of germ-free mice treated with amoxicillin-clavulanate after fecal microbiota transplantation from patients with idiosyncratic drug-induced liver injury

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Introduction/Objetives: Gut microbiota is related to the onset and progression of many liver diseases, but its role in idiosyncratic drug-induced liver injury (iDILI) by amoxicillin-clavulanate (AC) remains unknown. Our aim is to study the effect of gut microbiota (GM) from iDILI patients on the fecal metabolome in an animal model after fecal microbiota transplantation (FMT).
Materials and methods: Germ-free mice (GFm) were colonized with fecal microbiota from two patients with iDILI-AC (diDILI), two patients treated with AC who did not develop iDILI (dNOiDILI) and one healthy subject (dC). Three weeks after FMT, mice were divided in 3 groups per donor being treated with amoxicillin (A), AC or its vehicle. After 7 days of treatment, fecal samples were collected to evaluate GM composition and fecal metabolome. Moreover, correlation analyses were performed.
Results: The fecal metabolome analysis showed significantly altered pathways, highlighting arginine and tryptophan biosynthesis. Metabolites related to urea cycle like arginine, argininosuccinate and ornithine were decreased in diDILI/A and diDILI/AC groups compared to diDILI/H2O, trend not observed in C and dNOiDILI groups. Moreover, GM composition analyses presented specific altered genera between groups according to the donor. Additionally, negative correlations between metabolites related to urea cycle and certain genera, such as *Marvinbryantia*, *Howardella* and *Desulfovibrio* were observed, whereas *Parasutterella* correlated positively.
Conclusion: GM composition may play a role in GFm metabolomic status after FMT from iDILI patients following A and AC treatment, conditioning iDILI-AC onset and progression. However, further studies are needed.
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Key words: Drug-induced liver injury, gut microbiota, amoxicillin-clavulanate, metabolome

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Muscle physiology

P115 The role of Orai channels in regulating cholinergic contractions of the detrusor

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Store-operated Ca^{2+} entry (SOCE) is a well-established mechanism involved in the replenishment of sarcoplasmic reticulum (SR) calcium (Ca^{2+}) content in smooth muscle cells (SMCs). Reduction of [Ca^{2+}] within the SR is sensed by the luminal domain of Stromal interaction molecule 1 (STIM1) which activates plasmalemmal Calcium release-activated calcium channels (ORai). Ca^{2+} influx into the cytosol via ORai channels is then sequestered into the SR – restoring Ca^{2+} levels. Cholinergic-mediated contractions of bladder smooth muscle (Detrusor) rely on Ca^{2+} influx from the extracellular space (~70%) and Ca^{2+} release from the SR (~30%). The molecular candidates that facilitate SOCE and SR refilling in the bladder are poorly understood. The purpose of this study was to investigate the contribution of Orai channels to cholinergic-mediated responses of murine detrusor muscle.
Transcriptional expression of Orai subtypes was performed on RNA extracted from murine detrusor. qRT-PCR experiments reveal expression of Orai 1, 2 & 3, with Orai 3 mRNA levels two-fold higher than Orai1 & 2 (n=4). Isometric tension experiments were utilized to assess the contractility of detrusor strips. SOCE-mediated responses induced via thapsigargin were significantly reduced by the selective Orai channel inhibitor, GSK7975A (ANOVA, $p<0.0001$, n=6). Cholinergic-mediated responses were investigated using the Muscarinic receptor agonist, Carbachol (CCh). In the presence of GSK7975A, CCh-induced contractions of the detrusor were reduced from 8493 ± 616.6 mN to 5065 ± 602.3 mN (40% reduction, $p<0.0001$, n=6), and CCh-induced Ca^{2+} transients in isolated detrusor SMCs (n=5). Collectively, these results indicate that ORai channels play a significant role in mediating cholinergic-mediated responses of detrusor smooth muscle.
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Neuroscience and neurophysiology

P116 Cortical and hippocampal rhythmpathies in experimental models of brain metastasis evaluated with supervised learning tools

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Introduction/Objetives: Understanding the neurophysiology of brain tumours is critical to address the associated neurocognitive morbidities. Over recent years, several studies have shown alterations of neuronal network activity in peritumoral areas of primary brain tumours such as gliomas, with a bi-directional impact on cancer cells growth and on neural communication, which has been linked to hyperexcitability and seizures. However, the influence of brain metastasis on neural circuits remains relatively unexplored despite the high clinical prevalence of neurological symptoms. Here, we have evaluated microcircuit activity in the peritumoral microenvironment of mice models of brain metastasis generated by local injection of melanoma, breast and lung cancer cells.
Materials and methods: We used multi-site silicon probes in tumoral and contralateral areas of awake head-fixed mice to record the local field potential signals (LFP) from the cortex and hippocampus. Principal component analysis and different machine learning classifiers were applied to analyse electrophysiological data.
Results: Mice from melanoma, breast and lung cancer models presented a generalized reduction in the oscillatory power at different bands such as delta (1-4 Hz), theta (4-12 Hz) or ripple (100-200 Hz), and changes in the ratio between slow gamma (40-60 Hz) and fast gamma (70-90 Hz). Interestingly, we identified different LFP attenuation and tumour-type specific lateralized alterations that are not related with the size of the tumour mass. Instead, brain activity features matched with machine learning analyses confirmed model-specific alterations that could help to predict pathological electroencephalographic signals in advance.
Conclusion: Altogether, our data provide novel insights about the functional alterations associated with brain metastasis and highlight the use of predictive models for an early detection of brain metastasis signatures.
Key words: LFP, oscillations, brain metastasis, machine learning classifiers

P117 Mechanisms of LTP at Entorhinal Cortex Layer 3 - CA1 synapses

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Introduction and objetives: Synaptic plasticity appears to be involved in essential cognitive functions like learning and memory. Particularly, the hippocampus and the entorhinal cortex seem to be required for these processes. In addition, both areas are responsible for the processing of sensorial, spatial, and temporal information that may lead to the formation of short and long-term memory traces. Entorhinal Layer 3-CA1 (ECL3-CA1) synapses are relevantly involved in such processes and seem to be affected during the early stages of Alzheimer's disease.
Materials and methods: Juvenile C57BL/6 mice at postnatal (P) days 13-21 (P13-P21), were used to obtain 350 μm thick hippocampal slices. Electrophysiological recordings, including extracellular and intracellular whole cell patch-clamp recordings, were performed at ECL3-CA1 synapses. LTP was induced by applying a HFS protocol (100Hz, 1s). Excitatory postsynaptic potentials (EPSP) from CA1 area were monitored.
Results: A robust LTP was obtained when this protocol was applied. No changes in paired-pulse ratio were observed after the induction of this LTP, suggesting a postsynaptic locus of expression. This form of LTP was prevented when the slices were treated with the NMDAR antagonist D-AP5 or when the postsynaptic neuron was loaded with the NMDAR channel blocker MK-801. LTP was also prevented when the slices were treated with the mGluR antagonist LY341495 and with the mGluR5 antagonist MPEP. In addition, ECL3-CA1 LTP was prevented when postsynaptic neurons were loaded with the Ca2+ chelator BAPTA or when slices were treated with the L-type Ca2+ channel blocker nimodipine.

Furthermore, this form of LTP was prevented when the slices were treated with the CB1R antagonist AM-251, but was not affected when postsynaptic neuron was loaded with tetrahydrolipstatin. Finally, this form of LTP requires astrocyte activity to mediate D-Serine release as gliotrasmitter that is presumably activating postsynaptic NMDAR as it was lost when BAPTA was included into astrocytes and recovered when D-Serine was added extracellularly when astrocytes were loaded with BAPTA.
Conclusion: A HFS protocol (100 Hz,1s) induces LTP at EC L3-CA1 synapses in P13-P21 mice. This form of LTP requires postsynaptic NMDAR, mGluR5, postsynaptic Ca2+, CB1R , astrocytes and D-Serine.
Key words: Plasticity, electrophysiology, patch-clamp, long-term potentiation.

P118 Nimodipine inhibits ischemic neuropathology in mouse live brain slice preparations

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Introduction: Nimodipine is used to prevent delayed ischemic deficit in patients with aneurysmal subarachnoid hemorrhage (aSAH). Although nimodipine is primarily known as a cerebral vasodilator, it may have a more complex mechanism of action due to the expression of its target, the L-type voltage-gated calcium channels (LVGCCs) in various cells in neural tissue. This study was designed to investigate the direct effect of nimodipine on cerebral ischemic tissue injury and neuroinflammation.
Materials and methods: Spreading depolarization (SD), a pathomechanism of acute brain injury was induced in control or nimodipine-treated live mouse brain slice preparations under physiological conditions using electrical stimulation, or by subjecting the slices to hypo-osmotic stress or mild oxygen-glucose deprivation (mOGD). SD was recorded applying local field potential recording or intrinsic optical signal imaging. Histological analysis was used to estimate tissue injury, the number of reactive astrocytes, and the degree of microglia activation.
Results: Nimodipine did not prevent SD occurrence in mOGD, but it did reduce the rate of SD propagation and the cortical area affected by SD. In contrast, nimodipine blocked SD occurrence in hypo-osmotic stress, but had no effect on SD propagation. Furthermore, nimodipine prevented ischemic injury associated with SD in mOGD. Nimodipine also exhibited anti-inflammatory effects in mOGD by reducing reactive astrogliosis and microglial activation.
Conclusions: Nimodipine directly inhibits SD and neuroinflammation, and thereby provides neuroprotection. Our results suggest that the indication for clinical nimodipine administration may be broadened to target brain injury states where SD or neuroinflammation are thought to worsen outcomes.
Key words: cerebral ischemia, neuroprotection, nimodipine

P119 Neural networks for space, time, and number perception: a meta-analysis of neuroimaging studies

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Introduction/Objetives: Rapid and coordinated processing of space, time and number is fundamental for the daily flow of life. ATOM theory proposes these perceptions are processed in partially overlapping brain areas as a magnitude system. We aimed to combine the results of fMRI studies conducted on space, time and number perception using a coordinate-based meta-analysis approach, to show overlapping brain areas.
Materials and methods: We conducted three separate literature searches for space, time, and number perceptions in accordance with PRISMA guidelines. 19 articles for space perception, 38 for time perception, and 31 for number perception were identified as suitable for meta-analysis. Coordinates taken from the articles were subjected to meta-analysis through the " GingerALE" program using " Activation Likelihood Estimation" statistical method. A total of 7 meta-analysis results were obtained through single dataset and conjunction analyses.
Results: Fronto-parietal dominated activation maps were obtained in space, time and number perceptions; additionally basal ganglia activations were predominant in time perception. In double conjunction analyses, fronto-parietal and insular activations were noted. The triple conjunction analysis showed activations in the right inferior parietal, inferior frontal and anterior insular cortex.
Conclusion: Our results support that space, time and number perceptions occur through partially overlapping neural networks. The parietal and prefrontal cortex regions shown in the triple conjunction analysis were previously suggested as significant cortical areas in the ATOM theory. The insula is associated with interoception and salience and has been reported in previous studies related to space, time and number perceptions. Our results are in support of the ATOM theory.
Key words: ATOM, meta-analysis, magnitude

P120 Does the "Learning effect" affect auditory and visual attention?: A Preliminary study

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Introduction/Objetives: The aim of the study is to investigate how this situation affects auditory and visual attention separately by creating a learning effect.
Materials and methods: 86 people without any health problems were included in the study. Monkey Business Illusion(MBI) video was used for the visual attention task. In this video, there were three types of situations that required attention. For the auditory attention task, pure sounds at four different frequencies (500, 1000, 2000, 4000 and 6000 Hz) were used. These sounds were embedded and integrated into the video. To create the learning effect, the Inattentional Blindness (IB) video, which is a video similar to the MBI video, and the same pure sounds were added into the video.

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Participants were first shown the IB video and were informed about situations requiring audio-visual attention. Afterwards, the participants were asked to watch the MBI video and tell when they heard the visually changing situations and the sounds coming from behind.
Results: The effect of learning on visual attention tasks was not observed to be statistically significant. However, in the auditory attention task, a statistically significant difference was found only in noticing the 6000 Hz pure tone.
Conclusion: The rationale for this result may be related to the first factor Inattentional Amnesia. Inattentional Amnesia is lower awareness during a difficult task and suggests that it may be due to a decrease in the encoding of critical stimuli into memory, and therefore, forgetting irrelevant or less important information. Hearing the 6000 Hz pure sound has the highest frequency compared to other pure sounds presented and may have been remarkable because it was at an unusual frequency.
Key words: auditory attention, visual attention, pure tones

P121 Preliminary observations on the influence of corpus callosum subregional lesions on cross-education

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Introduction/Objectives: Cross-education (CE) is the physiological phenomenon by which unilateral muscle training increases strength and skills in the contralateral, untrained homologous muscles. CE is credited to be mainly mediated by interhemispheric interactions via corpus callosum (CC) projections between motor and premotor areas. However, the relationship between CC subregions connecting these areas and CE magnitude is still unknown. Therefore, we aimed at testing the relationship between lesions in CC subregions and CE magnitude, using multiple sclerosis (MS) as a model of CC damage.
Materials and methods: Nine persons with MS, displaying unilateral hyposthenia of the ankle dorsiflexion muscles (DF) and CC lesions, sustained a 6-week (3-days/week) maximal isokinetic concentric training of the unaffected DF. Muscle strength was assessed before and after training by measuring peak torque (PT) at 10°/s. Lesions' volume in each CC subregion was quantified on MRI scans by semi-automated segmentation and contouring techniques.
Results: PRE vs POST analysis showed a significant increase in PT in the untrained limb (+34.9%, $p<0.01$, $d=0.98$). Spearman correlations revealed a significant association between CE and anterior CC midbody lesion volumes ($\rho=-0.67$, $p=0.04$). No other significant associations were found.
Conclusion: Data confirmed CE occurrence despite CC damage. However, the CC lesional burden negatively impacted the magnitude of CE, confirming the hypothesis of CC involvement in strength transfer from the trained to the untrained side. Moreover, the correlations showed a potentially higher influence on CE magnitude of lesions located in the anterior midbody.
Key words: cross-education**,** corpus callosum, resistance training

P122 The correlation between P300 results and MoCA: a preliminary study

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Introduction/Objetives: The P300 is one of the electrophysiologic tests that allows the objective assessment of components such as attention and memory. MoCA is a popular and easy-to-administer test used to detect cognitive impairment. The objective of this study was to investigate the correlation between P300 latency and attention and memory scores obtained in MoCA.
Materials and methods: Twenty-one individuals with normal hearing between the ages of 20 and 40 were included in the study. P300 and MoCA test were applied to the participants. 1000 Hz and 2000 Hz stimuli were used as frequent and rare stimuli, respectively, and participants were asked to pay attention to the rare stimuli and count them. Electrode placement was made as non-inverting electrode Vertex (Cz), ground electrode (Fpz), and inverting electrodes as right and left mastoid. Stimuli presentation was performed with insert earphones. Attention and memory scores in the MoCA test were calculated separately. The Spearman correlation test test were used for analysis. The level of significance was taken as 0.05.
Results: Strongly significant negative correlations were found between P300 latency and attention and memory scores obtained from MoCA ($p<0.01$).
Conclusion: We found that as P300 latency increased, attention and memory scores in MoCA decreased. Considering the administration, cost and duration of both tests, it is thought that the current correlation may facilitate clinical use. Finally, our study is preliminary and further studies are needed to expand on the correlation between P300 and MoCA, especially involving a larger sample size.
Key words: attention, memory, MoCA, P300

P123 A distributed cortical network for the magnitude system of the brain: Insights from an fMRI study

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Introduction/Objetives: A theory of magnitude (ATOM), which proposes that spatial, temporal, and numerical magnitudes are perceived by a common mechanism located in the parietal and frontal areas, has some supportive as well as contradicting findings in the literature. The purpose of this study is to use fMRI to investigate whether there is a common neural mechanism for perceiving space, time, and number.
Materials and methods: Our design includes a visual reproduction task for three distinct modalities and a color reproduction task as the control condition. Participants (n=15, 7 female, mean age: 26.13, right-handed) are asked to judge the size, duration, number, or color of the stimuli and reproduce it by pressing a button. We collected neuroimaging data with a 3 T Siemens MAGNETOM Prisma MRI scanner while they perform the task and analyzed the data using

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SPM12. To investigate the common mechanisms, conjunction analysis was performed for space, time, and number task related activities.
Results: Statistical results were shown at the p<0.01 level, with a cluster-extent threshold of k≥10. Conjunction analysis revealed activations in the bilateral superior parietal lobule and left superior frontal gyrus.
Conclusion: Generally, it is thought that parietal areas are involved in the perceptual processes of magnitude, while frontal areas play executive functions. Our findings are consistent with the literature, suggesting a frontoparietal localization for magnitude system and providing support for the ATOM theory.
This study was conducted with the support of TÜBİTAK (The Scientific and Technological Research Council of Turkey) under project code [122K293].
Key words: fMRI, magnitude system, ATOM theory

P124 Distinct neural mechanisms in mental number line and arithmetic operations are not affected by mathematics anxiety

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Introduction/Objetives: The processing efficiency of spatial representations of numbers is thought to underlie arithmetic performance. Here, we aimed to investigate the brain activation differences in mathematics anxiety during the mental number line task and arithmetic operation task.
Materials and methods: Participants were 22 young adults consisting 11 with low mathematics anxiety (mean age: 21.00±3.10) and 11 with high mathematics anxiety (mean age: 22.91±1.64). Both groups showed significant differences in mathematics anxiety scores (t=-7.097, p<.001), as assessed using the Math Anxiety Rating Scale-Short Version. During fMRI scanning, participants performed two numerical tasks: a mental number line task and an arithmetic operation task. The functional images were acquired using a 3T MRI scanner and analyzed with SPM12. The time spent in education and exam anxiety were controlled as covariates. Activations were considered significant at p<0.05, after corrected with multiple comparisons using the family-wise error (FWE).
Results: The corrected results showed that the mental number line task activated parietal brain regions, including the bilateral supramarginal gyrus and right angular gyrus. In contrast, arithmetic operations engaged fronto-parietal brain areas, including the left intraparietal sulcus and left inferior frontal gyrus, along with the right secondary visual cortex, and right anterior insula. However, there were no functional brain differences in between the mathematics anxiety groups during numerical tasks.
Conclusion: The findings suggest that mathematics anxiety may not directly influence the neural processes involved in either the mental number line or arithmetic operations. On the other hand, the neural bases for number-space interaction and arithmetic are functionally segregated through distinct brain mechanisms.
This study was funded by the Health Institutes of Türkiye (TUSEB) with grant number 16469.
Key words: Numerical Cognition, Math Anxiety, fMRI

P126 Action observation in virtual reality: Does the perspective matter?

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Introduction: Action observation therapy (AOT) has traditionally been delivered on 2D screens using either first-person (lPP) or third-person perspectives (3PP). Immersive virtual reality (VR) offers a new way of delivering AOT, enhancing immersion and personalization. This study aims to examine the effects of these perspectives on motor excitability during AO in VR.
Materials and methods: Subjects observed a video of an avatar pressing a button in both lPP and 3PP, keeping their arm raised during the observation while high-density electromyography (EMG) signals were recorded from the anterior deltoid. Concurrently, electrocardiogram and respiratory movements were collected to compute heart rate variability (HRV). Perceived fatigue was evaluated administering the Borg scale; moreover, the subjective embodiment was evaluated by means oftwo additional scales (URGE and SENSATION of movement).
Results: While the EMG analysis <loes not revea! any significant difference in the myoelectric fatigue occurring at end ofthe contraction between lPP and 3PP, Borg scale seores are significantly lower in lPP. Moreover, in lPP slow events ofincreased EMG activity randomly occur more frequently. Additionally, HRV differences suggest a higher sympathetic activity when watching in lPP, the URGE and SENSATION scales revea! a greater embodiment for lPP.
Conclusion: The results on the EMG activity suggest that lPP may provide better access to motor areas increasing their excitability and reducing the perceived fatigue. Overall, lPP might be the preferential perspective for AOT, being able to elicit a higher motor involvement and a deeper engagement.
Key words: action observation therapy; virtual reality; motor excitability

P127 Interictal migraine reveals posterior insular and anterior cingulate cortex activation in response to high-frequency visual stimuli

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Introduction/Objetives: Migraine, a common neurological disorder with recurrent headache episodes, is actively researched for its relationship with visual sensory processing and cognition. Understanding migraine pathophysiology is crucial for developing effective treatments. This study

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examines neural alterations in visual processing during the interictal phase in migraine patients.
Materials and methods: Using a block design, this study investigated sensory sensitivity differences between migraine patients in the interictal phase and healthy controls. The sample consisted of 24 females (14 interictal, 10 controls) selected through clinical interviews. During fMRI scanning, participants engaged in visually demanding shape discrimination and localization tasks to assess sensory sensitivity. Images were obtained using a 3T MRI scanner and analyzed with SPM12.
Results: The main finding indicates increased activation in the posterior insula and anterior cingulate cortex in migraine patients during interictal periods compared to healthy controls during visual tasks. This suggests heightened sensitivity to visual stimuli during interictal periods. Additionally, the anticipation of pain from high-contrast images may have triggered activation in both the insula and anterior cingulate cortex. This hypothesis is based on the association of these brain areas with pain processing and the possibility that visually demanding tasks could elicit such responses.
Conclusion: Migraine patients exhibit heightened activation in the posterior insula and anterior cingulate cortex during visually demanding tasks in the interictal phase, indicating increased visual sensitivity. This activation likely arises from pain anticipation, as these brain areas are integral to the pain processing network. Understanding these neural changes can improve treatment strategies for migraine sufferers.
Key words: cingulate cortex, fMRI, insula, migraine

P128 Assessing the effectiveness of long-term biofeedback training in a patient with severe depression and moderate persistent bronchial asthma – a case report

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Introduction: It is well known that anxiety and depression may be associated with poor asthma control. It can be suggested that the reduction of depressive symptoms with long-term biofeedback training might have a positive effect on asthma in patients with such comorbidity.
Materials and Methods: A 24-year-old obese woman with severe depression and moderate persistent bronchial asthma, who developed social anxiety and had moderate panic attacks, went through an 11-week biofeedback training following the HeartMath protocol to reduce the symptoms of stress, anxiety, and depression. Before the training, she had daily stress-related asthma and panic attacks, which occurred at least three times per week. Her body weight was 92 kg. The woman also had high scores on the Perceived Stress Scale, Zung Self-Rating Anxiety Scale, Beck Anxiety Inventory, and Beck Depression Inventory. She had episodes of emotional eating every day. To objectify the symptomatology, we test immunoglobulin A and cortisol in saliva.
Results: After the training program, the patient did not have any stress-related and panic attacks for five weeks. Her weight was reduced to 85 kg, and her questionnaire scores decreased significantly. She quit emotional eating and has good, persistent eating habits. The levels of cortisol and IgA were tested before and after the training program, and they were also significantly reduced.
Conclusions: These findings suggest that long-term biofeedback training programs might be a reliable non-pharmacological approach to reduce the symptoms of stress, anxiety, and depression and to enhance associated somatic disorders such as asthma.
Key words: depression, bronchial asthma, biofeedback training

P129 Callosal oscillatory differences between fast and slow healthy adult readers

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Introduction: Neural oscillations in different frequency bands coordinate and synchronize word decoding and lexical accessing processes, enabling efficient and effective reading. The main objective of our study was to investigate the role of interhemispheric callosal network in reading, specifically focusing on the transfer times of different frequency bands.
Materials and Methods: We utilized EEG data of 51 right-handed fast and slow adult readers during high frequency word and pseudoword reading paradigm. We calculated the callosal transfer times on posterior electrodes (O1-2, P7-8) separately for alpha, theta, and beta frequency bands.
Results: Our ANOVA results revealed that IHTT in the alpha frequency band (18.395 ± 1.607 ms) was slower than both theta (13.057 ± 1.23 ms) and beta (15.357 ± 1.269 ms) bands ($p < 0.001$ and $p = 0.05$, respectively). According to the post hoc test, there was a trend toward a significant elongated RL transfer in slow readers for alpha frequency ($p = 0.055$).
Conclusion: Our observation aligns with literature. It suggests that the slower interhemispheric transfer time during word decoding in slow readers may be attributed to a neuronal synchronization problem caused by alpha oscillations. Considering the existing research on alpha oscillation and attention networks, we propose that this result indicates a difference in attention processes between the two groups. Our study sheds light on the importance of callosal network dynamics and alpha oscillations in reading, offering insights into the underlying neural mechanisms and potential attention-related differences between fast and slow readers.
Key words: alpha band, word reading, IHTT

P131 Estrogen: mechanisms of neuroprotective and neurotrophic effects

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Estrogen is an important steroid hormone signal that regulates multiple tissues and functions in the body. Classically, it is considered a reproductive hormone, due to its well-known role in feedback signaling in the hypothalamic-pituitary-ovarian axis. This review will focus on the "non-reproductive" effects of estrogen in the brain, specifically on the neuroprotective actions of estrogen. The brain is an important target organ for estrogen. In addition to direct effects, estrogen influences brain function through effects on the vasculature and the immune system. Estrogen influences several neurotransmitter systems, including acetylcholine, serotonin, noradrenalin, and glutamate. There are complex mechanisms that underlie estrogen neuroprotective and neurotrophic actions (genomic, non-genomic kinase signalic pathways and membrane estrogen receptors pathways). Estradiol induces gene transcription and

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rapid membrane signaling mediated by estrogen receptor alpha (ERα), estrogen receptor-beta (ERβ), and a recently characterized G-protein coupled estrogen receptor, each with distinct distributions and ability to influence estradiol-dependent signaling.Vector-mediated expression of estrogen receptors in the hippocampus provides an innovative research approach and suggests that memory depends on the relative expression of ERα and ERβ interacting with estradiol levels.The potential role of estrogen as a neuroprotective factor and a multicellular mode of estrogen action in the regulation of neuronal survival and neurotrophism is discussed, as are potential future directions for the field.

Key words: estrogen; steroid hormone; neuroprotection.

P132 In situ assessment of neurophysiology and neuropathology in a zebrafish model of ALS

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Introduction/Objectives: Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disease characterised by loss of motor neurons and motor function. ALS also affects other neural cells and often causes cognitive and behavioural impairment. ALS pathophysiology includes loss of proteostasis and oxidative damage. Previous studies proposed bisphenol-A (BPA) exposure as an environmental model of sporadic ALS (sALS) in zebrafish. Here, we aim to characterise this sALS model, connecting functional phenotypes with differentially-vulnerable cell types.

Materials and methods: We characterised the BPA (10-60 µM)-induced model of sALS in zebrafish (AB strain) at 0-6 days post-fertilisation, assessing motor/circadian activity, axon length, ER stress, and oxidative stress. To evaluate the model's predictive validity, we tested the effects of an approved ALS drug, edaravone (100 µM), on sALS phenotypes. To identify sALS-relevant vulnerable cells, we tested the combination of in-situ hybridisation with a new zebrafish line encoding a fluorescent reporter of protein stress.

Results: BPA reduces motor activity and shortens motor axons in a concentration-dependent manner. Further, BPA increases oxidative damage (ROS production and lipid peroxidation) and ER stress in whole zebrafish. Edaravone ameliorates BPA-induced motor and ER phenotypes. Lastly, using in situ hybridization and a zebrafish reporter line of protein stress, we show a proof-of-concept for identifying vulnerable cells.

Conclusion: BPA exposure induces sALS-consistent phenotypes in zebrafish. Edaravone counteracts BPA-induced sALS phenotypes, supporting the predictive validity of the model. The BPA-induced sALS model is useful for pathophysiological studies, contributing to fulfil a need for validated sALS models, and enabling the identification of differentially-vulnerable neural cells.

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Key words: ALS, zebrafish, bisphenol A, in situ hybridisation.

P133 Modulation of neuronal excitability by voltage and calcium in developing CA1 pyramidal neurons

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Introduction/Objetives: Neuronal excitability relies on the activation of gated channels. The hippocampus undergoes intense morphological and molecular changes during the first postnatal weeks of development in rats, including upregulation and downregulation of voltage-gated and ligand-gated channels. Here, we have studied how holding potential and extracellular calcium concentration modulate the action potential morphology and the spike afterpotentials (after-depolarization -ADP- and after-hyperpolarization -AHP-) in developing CA1 pyramidal neurons.

Materials and methods: We used whole-cell patch-clamp in current clamp configuration to record the intrinsic activity of CA1 pyramidal neurons from 2- and 3-weeks old rats.

Results: At hyperpolarized holding potentials (-80 mV), action potentials increased their width and amplitude along development. Furthermore, the action potential threshold became more hyperpolarized, and the spike ADP became more prone. Depolarizing the holding potential (-60 mV) broadened the action potentials and depolarized their threshold. Interestingly, action potentials were specially broadened in less mature neurons, accompanied by an increased amplitude. Moreover, neurons were more prone to fire in bursts at depolarized holding potentials, and they showed smaller mAHP amplitude. Extracellular calcium also affected both action potential waveform and spike after-potentials. However, some effects were calcium-specific (controlling spike repolarization and AHP) but others were due to its condition as a divalent ion (spike threshold). In summary, we found that voltage and calcium differentially modulate some intrinsic features of developing CA1 pyramidal neurons.

Conclusion: These data reflect a maturation of the intrinsic excitability in CA1 pyramidal neurons which would allow a more efficient processing of the stimuli.

Key words: Excitability, CA1, action potential, AHP, ADP, calcium

P134 Central and autonomic nervous system responses during geomagnetic storms.

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Introduction/Objetives: We tested the hypothesis that Geomagnetic storms (GMSs) as environmental stressors, will have different effects on humans' central and autonomic nervous systems (CNS/ANS) depending on the baseline type of ANS.

Materials and methods: Healthy males (n=30), aged 18-24, took part in the study. Heart rate variability (HRV) method and EEG frontal asymmetry index for α spectra were used to detect changes in the magnetically quiet periods and with GMSs occurring. For statistical analysis, t-test were used, with a 0.05 significant level.

Results: In the case of high vagal tone (HVT), changes reveal significantly increased RMSSD (the square root of the arithmetical mean of the sum of the squares of differences between adjacent NN intervals) (p=0.053), in the GMSs days compared with quiet days, which indicates the increased parasympathetic and reduced sympathetic activities (PS; SA) of ANS. There is also increased power of F3 compared with

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F4, (-0, 05396) indicating a little α left asymmetry i.e. relative right-sided frontal activity in GMSs days.
In the case of low VT (LVT), changes indicate a significantly reduced RMSSD (p=0.031), and HF (high-frequency of HRV spectra), (p=0.031), indicating reduced PA and increased SA of ANS, which coincided with significantly increased F3 compared with F4, (-0, 10084) indicating greater relative right frontal activity during GMSs.
Conclusion: The baseline HVT provides the optimal adaptation of healthy males to protect them from GMSs manifesting increased HRV and less FAI. Males with baseline LVT are more vulnerable to the impact of GMSs which is revealed in decreased HRV and greater relative right frontal activity during GMSs.
Key words: High vagal tone, Frontal asymmetry, Geomagnetic storms.

P135 Yeast Supplementation Potentiates Fluoxetine's Antidepressant Effect in Mice via Oxido-inflammatory, CREB, and MAPK Signaling Pathways

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Introduction/Objectives: The relevance of oxido-inflammatory, CREB and MAPK pathways in the pathophysiology of depression is known. Nutritional yeast has been suggested to have probiotic, antioxidant, and anti-inflammatory properties. However, the therapeutic potential of yeast in the management of depression is unknown. Thus, we evaluated the modulatory effect of nutritional yeast supplementation on antidepressant activity of fluoxetine in mice models of depressive-like behaviors (DLB).
Materials and Methods: A total of 112 mice were divided into 16 groups (n = 7 each) for a 3-stage study. Stage I (non-DLB study) had groups Ia (10 mL/kg vehicle), Ib (20 mg/kg fluoxetine), Ic – If (2% yeast diet for all, but Id - If additionally received 5 mg/kg, 10 mg/kg, and 20 mg/kg fluoxetine respectively). Stage II (lipopolysaccharide [LPS] model of DLB) had groups IIa - IIb (10 mL/kg vehicle), IIc (20 mg/kg fluoxetine), IId (yeast) and IIe (yeast + 20 mg/kg fluoxetine). After these treatments for 24 days, animals in IIb - IIe received 0.83 mg/kg of LPS on the 25th day. Except for group IIIa (10 mL/kg vehicle), animals in other groups of stage III (unpredictable chronic mild stress [UCMS] model) were exposed to UCMS for 24 days along with 10 mL/kg vehicle (IIIb), 20 mg/kg fluoxetine (IIIc), yeast (IIId), or yeast + fluoxetine (IIIe).
Results: Yeast and fluoxetine attenuated LPS- and UCMS-induced immobility, derangement of oxido-inflammatory (TNF-α, IL-6, NO, MDA, SOD, GSH, CAT, and AChE) and CREB/MAPK pathways. While fluoxetine had more potent effect than yeast when used separately, pre-treatment of mice with their combination had more pronounced effect than either of them.
Conclusion: Nutritional yeast supplementation improves the antidepressant activity of fluoxetine in mice by modulating oxido-inflammatory, CREB, and MAPK pathways.
Key words: Depression; Fluoxetine; Yeast.

P137 Activation of dopamine D4 receptor prevents morphine-induced impairment of adult neurogenesis in the subventricular zone: implications in odor discrimination learning

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Introduction/Objetives: Morphine reduces adult neurogenesis in the subventricular zone (SVZ) and therefore the integration of newly generated neurons in the local olfactory bulb circuit. Besides, altough it has been scarcely studied, some data suggests that opioid drugs interfere with olfactory perceptio, but the underlying mechanisms are unknown. We have previously shown that the activation of the dopamine D4 receptor (D4R) prevents both morphine addiction -by modulating nigroestriatal dopaminergic signaling- and the development of morphine-induced analgesic tolerance -by regulating the primary circuit of pain within the dorsal horn. Here, we hypothesize that the D4R could also counteract the impairment of olfaction associated with morphine.
Materials and methods: We investigated the effect of chronic administration paradigms of combined treatment of morphine and the D4R agonist PD168,077 on the SVZ neurogenesis in adult rats using immunohistochemistry as well as its functional implications by an olfactory discrimination test.
Results: In the SVZ, the D4R activation counteracted morphine-induced depletion of newly generated glial cells (astrocytes and oligodendrocytes) and neuroblasts as well as the increase of tyrosine hydroxylase expression. Besides, three weeks of chronic administration of morphine impaired olfactory discrimination between a pair of odorants, which was completely prevented by the co-administration with PD168,077.
Conclusion: The present results deepen in the dopaminergic regulation of olfaction and give support for the existence of antagonistic functional D4R-MOR interaction in olfactory bulb and SVZ that could help to the development of new pharmacology strategies for the treatment of pain reducing side-effects of morphine.
Key words (about 40 characters): Olfaction, morphine, dopamine D4 receptor

P138 Allostatic load in the context of war-related stress and transgenerational impact of former Yugoslavia conflict

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Introduction/Objectives: This study investigates the impact of war-related stress on civilians affected by the former Yugoslavia war and the transmission of this impact on a

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subsequent generation. Allostatic load is a novel concept for evaluation of the cumulative physiological burden of chronic stress that includes measures from cardiovascular, metabolic, and inflammatory domains. We focused on the comparison of the preliminary data on the allostatic load index with group demographics and psychological scores that were part of the extended study.

Materials and methods: A total of 138 participants living currently in Czechia enrolled up to date (1st gen. war survivors (G1): n = 60, 2nd gen. (G2): n = 25, control group: n = 53) were assessed for 12-parameter allostatic load index (ALI), basic demographics and psychological scores (PTGI, PCL-5, brief-COPE, SF-36, MSPSS and SWLS).

Results: We found significant differences in ALI between the control group (2.61 ± 1.91 SD) and G1 (4.28 ± 2.41 SD), and between G1 and G2 (1.93 ± 1.35 SD) but only in males. Both G1 and G2 groups each differ significantly from control in the PTGI scale total and subscales, no differences were found in other scores used. Within the control group alone, there was a strong positive correlation of ALI with the SF-36 Energy subscale, and a negative one with brief-COPE Emotion-Focused subscale. Within the G2 group, ALI had a strong negative correlation with the brief-COPE Emotion-Focused subscale and MSPSS overall and Family subscale. No significant correlation between ALI and questionnaire scores was found within the G1 group. There was also a significant difference between ALI and SF-36 trends between study groups, a negative trend in control, a positive in G1, and no trend in G2.

Conclusion: Substantial allostatic load differences between G1 and G2 groups are gender specific, while both score similarly higher on post-traumatic growth. G2 group differs in less perceived family support with increasing ALI. Higher ALI also correlates with coping strategies less aiming to regulate emotions in both G2 and control groups. Interestingly, the control group shows higher perceived energy with increasing ALI.

Key words: war, trauma, allostatic load

P139 Protective effects of regular aerobic and/or resistance exercise programs on sleep deprivation-induced cognitive impairment

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Introduction/Objetives: To investigate the potential protective effects of regular exercise programs on cognitive dysfunctions induced by rapid eye movement (REM) sleep deprivation. This study was supported by Trakya University (TÜBAP-2023/03).

Materials and methods: Wistar albino rats (n=28) were divided into four groups following baseline evaluations and cognitive tests (open field, object recognition, elevated plus maze): Control, Aerobic Exercise, Resistance Exercise, Combined (Aerobic + Resistance) Exercise. Aerobic exercise included 30 min swimming exercise whereas resistance exercise cmprised of stair climbing (3 sets, 5 repetitions a day). Rats in the combined group performed both aerobic and resistance exercises. Control group remained sedentary. After 6-week exercise period, cognitive tests were repeated. Then all groups underwent short term (i.e. 72-hours) REM sleep deprivation and cognitive test panel was repeated for the third time.

Results: Exercise groups showed higher locomotor activity compared to the control animals at the end of six-week exercise program. Resistance and combined exercise groups showed reduced anxiety-like behaviour. Object recognition test results were similar in all groups. The elevated plus maze data suggest that exercise can have a positive effect on anxiety levels. Increasing time spent in the open arms was associated with a significant decrease in anxiety level. Assessment of behavioral parameters in the open field supports the positive effects of exercise; significant differences were found between the exercising groups. Significant differences were found between the exercising groups in the object recognition test.

Conclusion: Various exercise programs may improve and/or show protective effects on cognitive deteriorations induced by sleep deprivation.

Key words: sleep loss, anxiety-like beaviour, object recognition

P140 The effect of chronic sleep restriction in adolescence on anxiety-like behaviour in adulthood: an experimental study

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Introduction/Objetives: Sleep restriction occurs when sleeping less than the amount of sleep needed to maintain optimal performance. Sleep deprivation may induce a state of increased anxiety and regular exercise may provide ameliorative effects. We aimed to investigate the adulthood effects of sleep restriction and exercise during adolescence.

Materials and methods: Wistar rats were randomly divided into 4 groups (n=10, each) at 28 days of age. In an attempt to mimic daily life, sleep restriction group had 4 hours sleep 4 days of a week and had regular sleep for the remaining three days. Exercise group performed swimming exercise 4 days a week, and combination group underwent both exercise and sleep restriction. Control group remained in home-cage. The intervention protocol lasted 4 weeks. Then, anxiety tests (elevated plus maze, hole-board) were performed. When the rats reached adulthood (after 8 weeks) anxiety tests were repeated.

Results: In the elevated plus maze test, frequency of entries into open arms was higher in the exercise group compared to control and sleep restriction groups (45.6±6.9, 20.3±4.1, 22.5±4.8, respectively; ANOVA $p<0.05$). All groups showed comparable results in adulthood anxiety tests. In hole-board test, number of looking-through holes was higher in the exercise group than in the control group (85.7±8.8, 44.9±7.8, respectively; ANOVA $p<0.05$). However, there was no significant difference during adulthood period.

Conclusion: In adolescence, sleep restriction increased anxiety-like behaviors, while exercise showed preventive effects. In adulthood, no residual effect was observed. The anxiety-like effects of sleep restriction during adolescence disappeared in adulthood.

Key words: sleep loss, swimming exercise, REM sleep restriction

P141 The emerging role of D4 receptors in preventing morphine tolerance through the regulation of transcription factor expression in the dorsal horn

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Introduction/Objetives: Morphine is one of the most effective analgesics used in the clinical management of pain. However, prolonged exposure to morphine results in a broad spectrum of adverse effects, including tolerance and addiction. Our prior findings indicate that the activation of the dopamine D4 receptor (D4R) prevents morphine addiction by modulating dopamine signalling in striatal regions involved in drug-habit formation and consolidation, without altering morphine-induced analgesia. Furthermore, it has been demonstrated that D4R in the terminals of primary nociceptors contributes to the antinociceptive effect of dopamine. In this study, we evaluate the impact of a combined treatment of morphine with a D4R agonist in the treatment of acute inflammatory pain, with a special emphasis on the development of analgesic tolerance.
Materials and methods: Sprague Dawley rats were administered morphine and/or PD168,077 (D4R agonist) over 8 days. On days 1 and 8, 15 minutes after drug administration, formalin was subcutaneously injected into the hind paw of the rats, and pain behaviour signals were subsequently evaluated. Immunohistochemistry was used to assed the expression of the transcription factors: cFos (related with pain response), P-CREB (linked to opioid tolerance) and Pax2 (GABAergic marker) in the laminae I and II of the dorsal horn.
Results: Animals chronically treated with morphine showed a clear nociceptive response and an up-regulation of both c-fos and pCREB expression (pain and tolerance markers, respectively) in the laminae I and II. However, only in a set of animals, the activation of D4R prevents the analgesic tolerance induced by morphine, which was associated with a down-regulation of c-Fos and pCREB expression in the dorsal horn.
Conclusion: The D4R activation prevents morphine induced analgesic tolerance, which correlates with a specific signature of transcription factor expression in nociceptive pathways in the dorsal horn. Thus, it opens up the possibility of using a D4R agonist in combination with morphine as a novel strategy for treating pain.
Key words: pain, opioids, dopamine

P142 7,8-dihydroxyflavone affects brain nitric oxide production in animal model of depression

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Introduction: Changes in nitric oxide (NO) signaling may have a negative impact on various processes in the brain, including neurotransmitter and neuroplasticity dysregulation, and thus participate in the pathophysiology of depression. 7,8-dihydroxyflavone (7,8-DHF) is a natural flavonoid that mimics the effect of brain-derived neurotrophic factor (BDNF). 7,8-DHF possesses antioxidant properties, might influence the production of NO and reduce the expression of inflammatory markers. The aim of this study was to determine the effect of 7,8-DHF on nerve tissue changes related to pathological behavioral symptoms in a chronic unpredicted stress (CUS)-induced model in rats.
Materials and methods: 12-week-old male Sprague Dawley rats were divided into 4 groups: control rats; rats exposed to stressors; control and CUS-rats that received 7,8-DHF (5 mg/kg/day) every 3 days for 3 weeks. We used forced swim test (FST) and the elevated plus maze (EPM) test to detect behavioral changes. NO-synthase (NOS) activity, protein expression, and concentration of conjugated dienes (CD) were measured.
Results: In FST, we found higher immobility in CUS group. CUS resulted in reduction of NOS activity, decrease in nNOS and BDNF expression in the medial prefrontal cortex (mPFC) as well as increase in iNOS expression in the hippocampus. 7,8-DHF increased expression of nNOS, BDNF, TrkB and SOD as well as decreased iNOS expression. The concentration of CD was increased after CUS, while 7,8-DHF reduced CD concentration.
Conclusion: Our results suggest that increased oxidative state in the brain and reduction of BDNF production may play an important role in the pathophysiology of depression. 7,8-DHF has been shown to have both antidepressant and antioxidant effects.
The study was supported by grants: VEGA 2/0025/23 and VEGA 2/0122/24.
Key words: 7,8-dihydroxyflavone, NO, depression

P143 Foreign first-year female medical students do experience acute stress in the new environment as evidenced in a virtual model

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Introduction/Objetives: The aim of the study was to investigate foreign (F) junior medical students stress due to new education environments.
Materials and methods: Sensorimotor reaction times (SMRT) measurement is an important tool for assessing a person's adaptation to stress. A study was conducted on F-(n=13) and local (L)-(n=11) first-year healthy female medical students. Participants were exposed to two randomized order simulated virtual visual tasks using simple (S) and discrimination (D) SMRT (SSMRT/DSMRT).
The correct answers (CA%), means (M), standard deviations (SD) and residual standard deviations (RSD) during SSMRT/DSMRT for both groups (F-1; L-2) were calculated.
Results:
M-SCA1= 85.9%; SD- SCA1=10.7; RSD-SCA1=2.964;
M-SCA2= 86.6%; SD-SCA2=4.3; RSD-SCA2=1.616;
M-DCA1= 50.2%; SD-DCA1=7.2; RSD-DCA1=1.987;
M-DCA2= 54.6; SD-DCA2=4.9; RSD-DCA2=1.85.
M-SSMRT1=0.352s; SD-SSMRT1=0.1077; RSD-SSMRT1=0. 02988; M-DSMRT1=0.7092s;
SD-DSMRT1=0.1476; RSD-DSMRT1=0.04094;
M-SSMRT2=0.3519s; SD-SSMRT2=0.09406s; RSD-SSMRT2=0. 03555; M-DSMRT2=0.6403s; SD-DSMRT2=0.2219; RSD-DSMRT2=0.08388.
There were no statistically significant differences between the different means for CA. Although, the SD/ RSD-SSMRT in F-students was much higher than in L-students. This indicates some neuroticism experienced by F-students, appearing in hyperactive responses.
There were no statistically significant differences between the different means for RT. The calculation of the Pearson correlation-r and variation coefficients - (CV=SD/Mean) revealed: r=0.7552; CV-SSMRT1=0.305966; CV-

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DSMRT1=0.208122; CV-SSMRT2=0.267292; CV-DSMRT2=0.346556.
There was a linear relation between the means and SD, and higher CV DSMRT for L-students indicating less concentration of attention in L-students.
Conclusion: The parameters of mental chronometry in F/L junior female medical students are the same. Some neuroticism in F-students can be explained by the presence of certain stress related to the difficulty adapting to a new environment. However, F-students can concentrate better than l-students on discrimination tasks.
Key words: First-year female medical students stress, Mental chronometry, Sensorimotor reaction time tasks.

P144 Investigation of the effects of agomelatine on appetite metabolism

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Introduction/Objetives: Hypothalamic nuclei synthesize anorexigenic and orexigenic neuropeptides. Melatonergic receptors (MT_1/MT_2) are localized in the hypothalamus where appetite-regulating factors are secreted. Agomelatine is a potent agonist of the MT_1/MT_2 receptors and is used as an antidepressant medication. This study aimed to investigate the effects of agomelatine on appetite metabolism.
Materials and methods: Forty male *Sprague Dawley* rats were divided into four groups: Control, Sham, Ago-20 and Ago-40. The control group received no treatment. During the 14-days, the sham group was given a vehicle, while the Ago-20 and Ago-40 groups were administered two different doses of agomelatine (20-40 mg/kg) by oral gavage. At the end of the treatment period, rats were euthanized and hypothalamic tissue samples were obtained. Hypothalamic AgRP, POMC, CART and NPY gene expression levels were assessed by RT-PCR and protein levels were evaluated by western blot analysis.
Results: The agomelatine-treated groups showed increased gene expressions of POMC, AgRP and NPY. Conversely, there was a decrease in the expression of CART gene ($p<0.05$). Furthermore, these groups exhibited increased protein levels of POMC and CART, while showing decreased protein levels of AgRP and NPY ($p<0.05$).
Conclusion: These findings indicated that agomelatine affects in the central regulation of appetite.
Keywords: agomelatine, POMC, CART, NPY, AgRP
This research was supported by The Scientific and Technological Research Council of Türkiye (Project no: 222S915).

P145 Impact of S100A10/P11 downregulation on spatial learning, memory, and long-term potentiation in CA3-CA1 synapses of the mouse

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Introduction/Objetives: Recently, we have communicated for the first time that P11 (S100A10) regulates neurotransmission at excitatory synapses onto hypoglossal motoneurons through presynaptic mechanisms by interacting, at least, with the molecular machinery for neurotransmitter release (IBRO Neurosci Rep 2023;15:S345; accompanying abstract by Vilches et al.). Here, we investigated whether P11 down-expression impacts on synaptic strength and plasticity at CA3-CA1 synapses of the hippocampus and how it can affect spatial learning and memory in adult mice.
Materials and methods: Spatial learning and memory studies were performed using the Morris water maze test (MWM) and new object recognition (NOR) in mice treated with cRNA or small interfering RNA against p11 (siRNAp11). These mice were then induced to undergo long-term potentiation (LTP) at the CA1-CA3 synapse in the hippocampus.
Results: p11 silencing in the hippocampus by means siRNAp11 induced a strong reduction in mRNA for p11 that was accompanied by an improvement in spatial learning and memory as evaluated by the MWM test. Differences in MWM outputs were accomplished exclusively by the male cohort suggesting sex-dependence of siRNAp11–induced alterations in this experimental paradigm. However, episodic memory, evaluated by the NOR test, was unaffected in the same cohorts of treated animals. Electrophysiology recordings performed in the same mice subjected to the behavioral tests showed that p11 silencing strongly reduced amplitude and slope of the field excitatory postsynaptic potential (fEPSP) at CA3-CA1 synapses and fully prevented induction of LTP. Strikingly, siRNAp11-induced alterations in fEPSP amplitude and LTP induction were also sex-dependent. Finally, p11 knockdown was accompanied by a robust decrease in the expression of the vesicular glutamate transporter-1 (vGLUT1) in the hippocampus.
Conclusion: These results signal P11 as a pivotal partner in spatial learning and memory by, presumably, impacting on synaptic strength and plasticity in the hippocampus.
Funding: MICIU (PID2019-110960GB-I00).
Key words: sex-differences, synaptic plasticity, vGLUT1.

P146 Effect of cold water face immersion test after exercise on reaction time

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Introduction/Objectives: Face immersion in cold water stimulates the autonomic nervous system and causes physiological changes such as apnea, decrease in heart rate and increase in blood pressure. These hemodynamic changes can be measured during various exercises. We aimed to investigate the effects of the cold water face immersion test (CWFIT) on reaction time after exercise. We also planned to analyze heart rate variability (HRV) to evaluate the effect of the autonomic nervous system on heart rate and to determine its neurogenic profile.
Materials and methods: Eighteen healthy young adult volunteers underwent a series of experiments. Blood pressure, heart rate, heart rate variability and reaction time were measured under three different conditions: at rest, following a 6-minute walk test (MWT), and after CWFIT. For CWFIT, each volunteer performed a 6-MWT and then immersed his/her face into cold (10°C) water for 15 seconds. Cardiac parameters and reaction times were compared among the three conditions by Freidman test.

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Results: There was no statistically significant difference in reaction times of three conditions. In HRV analysis, the low frequency high frequency ratio (LF/HF), which indicates sympathovagal balance at rest, following MWT and after CWFIT were 1.51±1.22; 6.35±5.35; 2.67±3.66 respectively. LF/HF increased significantly after exercise compared to rest ($p<0.05$). LF/HF decreased significantly after CWFIT compared to post-exercise ($p<0.05$).
Conclusion: In this pilot study, reaction time was not affected by exercise or CWFIT despite some alterations in sympathovagal balance observed in HRV.
Key words: heart rate variability, autonomic nervous system, diving réflex

P147 Influence of piracetam and diazepam on neocortically-induced seizure activity

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Goal: The goal of the study was investigation of the neocortical seizure reactions in the slow wave sleep and after injection of the pharmacological substance
Methods: Experiments were caried out in the wistar rats. Under the ketalar aenethesia bipolar and unipolar electrodes were implanted into the hippocampus and neocortex. Brief electrical stimulations of the dorsal hippocampus induced the focal epileptiform discharges. Piracetam (20,0-25,0 mg/kg) and Diazepam (0,5-1,0mg/kg) were injected intraperitoneally.
Results and conclusión: In the first series of experiments it was found that stimulation of neocortex produced longer epileptiform discharges during wakefulness than in the slow wave sleep, suggesting decreased epileptiform threshold of neocortex. After piracetam injection, stimulation of neocortex produced shorter seizure discharges. Same effect was found in a case of diazepam; However, Diazepam decreased both neocortical and hippocampal seizure reactions that suggested increase of epileptiform threshold in the neocortex and hippocampus. The results obtained are due presumably to influence of elevated GABA-ergic inhibition and increase of inhibitory influences of antidromic collaterals in the hippocampus.
Key words: dorsal hippocampus; neocortex, epileptiform discharges, piracetam, diazepam, sleep-wake cycle

P148 The effect of 7,8-dihydroxyflavone in the model of depression: differences between acute and chronic administration

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Introduction: 7,8-dihydroxyflavone (7,8-DHF) is a small-molecule ligand of tropomyosine kinase B (TrkB) with a pharmacodynamic properties similar to brain-derived neurotrophic factor (BDNF). The aim of this study was to compare acute and chronic 7,8-DHF administration on neurobilogical and behavioural variables in the model of depression.
Materials and methods: We used 12-week-old male Sprague Dawley rats (SD), which were exposed to unpredictable chronic mild stress (CUS) for 3 weeks. SD rats were divided into four groups: control gorup, control + 7,8-DHF, CUS group, CUS + 7,8- DHF. We used forced swim test (FST) to detect behavioral changes. NO-synthase (NOS) activity, protein expression, and concentration of conjugated dienes (CD) were measured.
Results: We found higher immobility in CUS group in both, acute and chronic experiments. 7,8-DHF decreased immobility in FST only after acute administration. CUS led to reduction of NOS activity in mPFC in both experiments, however 7,8-DHF increased NOS activity only in the acute experiment. Our results showed that CUS resulted in a decreased nNOS expression in mPFC in both experiments. Chronic administration of 7,8-DHF increased expression of nNOS, BDNF and SOD in the mPFC, while the expressions of these proteins remained unchanged in the acute experiment. The concentration of CD was increased after CUS, while 7,8-DHF reduced CD concentration in both experiments.
Conclusion: Exposure to chronic unpredictable stress led to a decrease in NO and BDNF levels as well as an increase in inflammatory markers. 7,8-DHF showed an antidepressant effect by upregulating the TrkB/BDNF signaling pathway, and also an antioxidant effect by reducing free radicals levels.
Supported by grants APVV-22-0271 and VEGA 2/0122/24
Key words: unpredictable chronic mild stress, 7,8-DHF

P149 Evidence for a synergistic effect of NPY1R agonist co-administered with Ketamine in rats: relevance to neurodegenerative and mood disorders

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Introduction/Objetives: Major depressive disorder is one of the most prevalent health problems worldwide and its current therapeutic approach still generates great dissatisfaction due to its limited efficacy, unwanted side effects and delays in effectiveness. This research addresses the problem by studying the combined effect of co-administration of Neuropeptide Y1R (NPY1R) and Ketamine on its neurobiological action in the hippocampus and its implications on cognition and mood-related behaviours.
Materials and methods: In the protocol used, the NPY1R agonist is administered intranasally together with ketamine intraperitoneally in adult Sprague-Dawley rats. Mood-related behaviours were assessed with the Forced Swimming Test (FST), while the object-in-place task was used for spatial memory. To examine markers of neurogenesis, in situ Proximity Ligation Assay, Immunohistochemistry for PCNA,

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DCX, BDNF and NPY1R/TrkB heteroreceptor complexes were performed in the hippocampus.
Results: Increased formation of NPY1R/TrkB heteroreceptor complexes and the presence of BDNF following co-administration has been found to be associated with improved memory consolidation, increased neuroblast proliferation and correlates with decreased immobility in the FST.
Conclusion: The formation of NPY1R/TrkB heteroreceptor complexes in the hippocampus by the synergistic effect of co-administration of NPY1R and Ketamine, improves cognitive functions, enhances the antidepressant effect, induces TrkB signalling and hippocampal neurogenesis. These findings open new therapeutic avenues for the treatment of neurodegenerative and mood disorders.
Key words: Neuropeptide Y1R Ketamine interaction, Neurodegenerative and Mood Disorders, Neurogenesis

P150 Investigation of Electrical and Behavioral Properties of Dentate Gyrus Prodynorphin Neurons in Amyloid Beta-Induced Alzheimer's Model Transgenic Mice

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Introduction/Objectives: Alzheimer's Disease (AD) is characterized by cognitive decline and the accumulation of abnormal proteins in the brain. The endogenous opioid system includes prodynorphin neurons which produce dynorphin peptides. Endogenous opioid peptides are involved in various physiological and psychological processes. Today, the role of the opioid system in AD and dementia mechanisms remains unclear. Therefore, we aimed to investigate a potential relationship between AD and prodynorphin neurons in the dentate gyrus (DGPdyn), a crucial area for learning and memory.
Materials and Methods: The potential roles of DGPdyn neurons in amyloid beta (Aβ) 1-42-induced male Pdyn-Cre transgenic mice models were investigated by using electrophysiological (patch-clamp), chemogenetic and optogenetic methods. Behavioral tests performed on the mice to analyze locomotor activity, anxiety-like behaviors, the short- and long-term memory states and the spatial memory/learning.
Results: Chronic inhibition of DGPdyn neurons in the PBS group caused a significant decrease in locomotor activity, while the activation of these neurons chemogenetically in the Aβ group reduced the velocity ($p<0,001$) and the novel object exploration duration time for both short-term memory ($p<0,01$) and long-term memory ($p<0,05$). Also, the chronic inhibition of these neurons improved spatial learning and memory, which were impaired in the Aβ-GFP group ($p<0,01$). Finally, the firing frequency of DGPdyn neurons increased significantly in Aβ-induced mice ($p<0.01$).
Conclusion: These results suggest that Aβ accumulation may disrupt locomotor-related synaptic transmissions of DGPdyn neurons by altering prodynorphin signaling. Furthermore, with an increased DGPdyn electrical activity in AD-like modeling, these neurons may be involved in the progression of AD by inducing cognitive impairment.
Key words: Alzheimer's Disease, Prodynorphin Neurons, Opto- & Chemo-genetics.

P151 Effect of acute sleep restriction on cardiovascular responses muscle metaboreflex activation in healthy women

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Introduction/Objectives: Metabolites accumulated in muscle during exercise stimulate muscle metaboreflex, which increases sympathetic nerve activity and blood pressure. Abnormalities in muscle metaboreflex~~es~~ have been associated with increased cardiovascular risk. In this study, we investigated the effect of acute sleep restriction on cardiovascular responses to muscle metaboreflex.
Materials and methods: Nineteen healthy young female volunteers underwent a series of experiments after habitual sleep and one-night sleep restriction (4 hours less than usual). First, volunteers rested for 10 minutes. To isolate muscle metaboreflex activation, they performed isometric hand grip (IHG, 2-min) at 30% of maximum voluntary contraction followed by post exercise ischemia (PEI, 2-min 15-sec) by inflation (200 mmHg) of a cuff placed around the same arm. After 20 minutes, the cold pressor test (CPT), as nonexercise sympathoexcitatory stimulus, was implemented. Blood pressure and heart rate were measured at rest, at the end of IHG, PEI and CPT, and heart rate variability (HRV) analysis was performed throughout the entire experiment.
Results: In IHG, PEI and CPT, both mean arterial pressure and low frequency high frequency ratio (LF/HF, indicating sempatovagal balance) of HRV analysis increased compared to rest under conditions of habitual sleep and sleep restriction ($p<0.05$ for all). However, muscle metaboreflex activation was comparable between sleep restriction and normal sleep conditions.
Conclusion: In this pilot study, acute sleep restriction resulted no alterations in muscle metaboreflex activity and cardiovascular responses.
Key words: exercise pressor reflex, isometric exercise, blood pressure

P152 Combination of sex hormones and transcranial magnetic stimulation to treat experimental autoimmune encephalomyelitis

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Introduction/Objetives: Multiple sclerosis (MS) is a chronic autoimmune, inflammatory, and neurodegenerative disease that affects the central nervous system, which causes oxidative stress in nervous organs. This disease affects women more than men, with a ratio of 3:1. Although the frequency in women is higher than in men, women have a milder course of the disease. The neuroprotective effect of sex hormones has been tested in preclinical and clinical trials, but the results are not conclusive. The aim of this study was to test the effect of

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estrogen (E), progesterone (P), and testosterone (T) hormonal supplementation as adjuvants to transcranial magnetic stimulation (TMS) treatment of experimental autoimmune encephalomyelitis (EAE) in rats, an experimental model of MS. Likewise, replacement therapy with these hormones was evaluated in EAE ovariectomized rats, treated with TMS.
Materials and methods: 65 female Dark Agouti rats were divided into Control; Vehicle; EAE; EAE+Mock; EAE+TMS; EAE+TMS+P; EAE+TMS+E; EAE+TMS+T; EAE+TMS+Sham; EAE+TMS+ovariectomy; EAE+TMS+OVX+P; EAE+TMS+OVX+E y EAE+TMS+OVX+T. All treatments were started at the same time, starting on day 14 after EAE induction. All treatments lasted 21 days. The glutathione redox cycle and biomarkers of oxidative stress were analyzed in brain and spinal cord.
Results: TMS alone is effective in reducing the oxidative stress that occurs in this disease. However, the use of estrogen and progesterone replacement therapy may enhance the beneficial antioxidant effects of TMS in ovariectomized rats with EAE.
Conclusion: The use of TMS in menopausal women with MS may be more effective if used in combination with hormone replacement therapy.
Key words: Estrogen, Hormone replacement therapies, Multiple Sclerosis

P153 Tick-tock delusion: the effects of short-term sleep restriction on subjective time perception in healthy women

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Introduction/Objetives: The significance of sleep in regulating various physiological processes and maintaining overall health is paramount. However, studies have shown that lack of adequate sleep duration and quality negatively affects cognitive functions such as memory, recall, and time perception. This study aimed to investigate the changes in time perception under conditions of short-term sleep restriction using retrospective and prospective tasks.
Materials and methods: After ethical approval, 13 healthy female volunteers, aged between 18 and 35 years, were included in this study. Time perception tests were performed following four consecutive nights of habitual sleep and repeated after three nights of sleep restriction (2 hours less than regular). The time perception tests comprised of retrospective time estimation (RTP, book reading and star-drawing tests) and prospective time generation tasks (PTP, time estimation using a reaction time device). To assess cognitive functions, the Stroop test, which evaluates selective attention, and the Wechsler Memory Scale-III (WMS-III), which measures short-term and working memory, were administered.
Results: The RTP after sleep restriction showed a significant prolongation compared to habitual sleep duration (21.6±8.4 vs 30.3±13.9 sec, respectively; ANOVA $p<0.05$). However, PTP was not affected by sleep restriction. Short-term and working memory performances decreased after sleep restriction (9.7±1.1 vs. 8.8±1 sec and 10.7±1 vs.10±1.3 sec, respectively; $p<0.05$ for both).
Conclusion: The prolonged retrospective time estimation and impaired memory performance following sleep restriction highlight potential risks for high-stakes work situations. Adequate sleep is essential for maintaining cognitive performance and accurate time perception.
Key words: sleep curtailment, time estimation, cognitive performance.

P154 Tau-Induced Perineuronal Net Degradation in Alzheimer's Disease

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Introduction/Objetives: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of extracellular amyloid beta (Aβ), and intracellular hyperphosphorylated tau that manifest as neurofibrillary tangles (NFTs) and tightly correlate with clinical presentation. The brain's extracellular matrices (ECM), particularly the perineuronal nets (PNNs), play a crucial role in brain functioning and neurocircuit stability, and reorganization of these unique PNN matrices has been associated with the progression of AD and accumulation of NFTs. However, the mechanisms driving these changes remain unclear.
Materials and methods: We utilized terminal tau-transgenic Wistar Kyoto (WK) rats, WK72, and their age-matched wild-type (WT) controls. We examined the brainstem (BS), where tau pathology primarily develops, via immunohistochemistry (IHC) using Wisteria floribunda agglutinin (WFA) and aggrecan, comparing the signals between the two groups. To further investigate the effects of tau propagation, we inoculated human brain-derived insoluble tau from histopathologically verified AD patients, as well as PBS, into the CA1 region of the hippocampus of WK72 rats. Subsequent IHC analysis was performed for PNNs (using WFA and aggrecan), phospho-tau Ser202 and Thr205 (using AT8), microglia/macrophage-specific calcium-binding protein (using Iba1), and astrocytes (using GFAP).
Results: We found a reduction in the amount of PNNs in BS of WK72 rats compared to their WT controls. Following hippocampal inoculation of human brain-derived tau aggregates in the CA1 region, the PNN signal was further reduced. However, there was no significant reduction in the number of pyramidal neurons throughout the hippocampal formation. Additionally, no significant reactivity of microglia or astrocytes was observed in the hippocampus in either group.
Conclusion: Tau pathology is associated with the reduction of PNNs. Understanding the mechanisms by which tau interacts with PNNs may offer new therapeutic targets for treating neurodegenerative diseases. Preventing PNN degradation or restoring their function could mitigate the adverse effects of tau pathology, providing a promising new direction for Alzheimer's disease treatment strategies.
Key words: Tau; Alzheimer's disease; perineuronal nets

P155 Alcohol and cannabinoid effects on neuroinflammation in adolescence rats

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Introduction/Objetives: During adolescence, alcohol and cannabis are the most-used (legal and illegal) substances,

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with a common dual consumption. Despite few data available, this dual consumption can induce neuroinflammation. Activation of glia has negative consequences on synaptic plasticity and brain function, increasing the risk of mental problems. Our aim was to evaluate the neuroinflammatory consequences after alcohol and cannabis treatment in adolescent rats.
Materials and methods: Sprague-Dawley rats were exposed to alcohol and Δ-9-tetrahidrocannabinol (THC, cannabis main psychoactive component) every 3 days during 1 month from 35 post-natal day (equivalent to human adolescence). One group had 5% (v/v) of alcohol in drinking water for 24h, another group was treated with THC (1,5 mg/kg, i.p.), a third group received both treatments, and controls were similarly manipulated but without drug exposure. After treatments, brains were perfused and sliced with a cryostat. Hippocampal slices were processed conveniently by immunofluorescence (IF) using antibodies against GFAP and NeuN, as glia and neuron markers.
Results: Analysis of GFAP expression revealed different patterns for alcohol and THC groups. Alcohol tends to increase microglia, and no changes were observed after THC treatment. Our preliminary results show that the increase seen after alcohol seems to be affected by THC when drugs were given combined. Fluorescence staining against NeuN shows that the increase of microglia after alcohol exposure goes along with a non-significant neuronal decrease.
Conclusion: Our preliminary results investigating the effects of alcohol and cannabinoid during adolescence in rats show neuroinflammatory process only after alcohol exposure; while changes were not observed on the THC treated brains.
Key words: cannabinoid, alcohol, adolescence

P156 Observation of isometric actions do not evoke motor resonance: a TMS study

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Introduction: In literature, most studies indicate activation of the mirror neuron system (MNS) when observing motor acts with visible movement.
To date, no studies have demonstrated the activation of MNS in response to the observation of isometric actions. This study aims to determine whether the observation of overt movement is essential for inducing motor resonance.
Materials and methods: The modulation of motor evoked potentials (MEPs), elicited by transcranial magnetic stimulation (TMS), was investigated in two distinct experiments.
Firstly, MEPs modulation was tested during the observation of two videos: in one a person holds a crate of water (isometric condition, ISO) and in the other the observed person keeps the exact same position without any muscle activity (resting condition, REST). Secondly, MEPs modulation was assessed during the observation of videos showing a subject's arm squeezing a soft ball, both immediately after the onset of the video (T1_ISO) and ten seconds later (T2_ISO).
Results: No significant differences were found between ISO and REST conditions, nor between T1_ISO and T2_ISO conditions, suggesting that a visible movement is necessary to elicit motor resonance. Results from the second experiment exclude the possibility that the lack of motor resonance when observing an isometric action lasting for several seconds is due to a reduced involvement of the MNS due to adaptation.
Conclusion: Overall, the present study suggests that mirror neurons do not respond to the observation of isometric actions.
Key words: Mirror Neuron System; isometric contraction; MEPs

P157 Maternal immune activation model variability: Unmasking the role of poly(I:C) weight

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Introduction: Maternal immune activation (MIA) models using poly(I:C) are vital for studying the effects of pregnancy inflammation and are used as prenatal models of psychiatric disorders. However, these models show inconsistent results. Commercially available poly(I:C) contains unknown ratios of low (LMW) and high (HMW) weight variants, which recent research suggests trigger different maternal immune responses.
Materials and methods: We investigated how MIA induced by HMW and LMW poly(I:C) (10 mg/kg) affects offspring's early development, adult social interaction, sensorimotor gating (PPI), and emotional processing. We also assessed maternal body temperature changes in response to treatment, litter sizes, and offspring body weight at postnatal days 13 and 21.
Results: While both LMW and HMW group offspring displayed significant behavioral deficits, some were uniquely displayed by one treatment group or sex. Notably, HMW females showed deficits in homing behavior and weaker sensorimotor gating (decreased PPI), while LMW females showed no such deficits. In contrast, LMW males displayed a broader spectrum of behavioral deficits than HMW males, including early motor deficits (negative geotaxis), stronger sensorimotor gating (increased PPI), and altered emotional processing. We found no significant differences in maternal body temperature, litter sizes, or body weight in pups.
Conclusion: These findings highlight poly(I:C) molecular weight as an under-considered factor in MIA model variability. Precise reporting of poly(I:C) weight may aid in standardizing models and improving our understanding of prenatal immune challenges on offspring development.
This project was supported by Czech Science Foundation grant GACR 22-08045S, GACR 22-15096S, and GAUK No 379121.
Key words: poly(I:C), maternal immune activation, animal behavior

P158 The effect of Insulin-like growth factor 2 on oxidative and neuronal stress in models of Parkinson's disease

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Introduction/Objetives: Mitochondrial dysfunction and oxidative stress are believed to play an important role in dopaminergic neurotoxicity in Parkinson disease (PD). Insulin-

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like growth factor II (IGF-II) might have neuroprotective and antioxidant effects. The main objective of this study is to examine the effect of the administration of IGF-II in two models of PD.
Materials and methods: The study of the cellular model focuses on the analysis of the effects of IGF-II on neuronal survival and neurodegeneration, dopaminergic markers, mitochondrial homeostasis, the intracellular pathways involved, in addition to the role of rIGF-II in the resulting effects. Its action on the dopaminergic pathway, astroglia and neuroinflammation is analysed in the mouse model.
Results: IGF-II prevents cell death and neurodegeneration and maintains morphology of the cell. It also assists in the recovery of the cellular markers associated with dopaminergic neurotransmission studied. IGF-II leads to a reduction in oxidative damage in mitochondria, with the recovery of antioxidant enzymatic activity, its functionality and morphology. The intracellular antioxidant NRF2 pathway and some of its dependent enzymes are recovered, as is the mTOR pathway. In the mouse model, administration of IGF-II blocks the loss of motor coordination and the reduction of self-grooming. IGF-II counteracts the neurodegenerative and pro-inflammatory effect on the nigrostriatal dopaminergic pathway.
Conclusion: Treatment with IGF-II in models of PD is capable of reversing the harmful effect of neurotoxicants on dopaminergic neurons, mainly thanks to the interaction with its specific receptor rIGF-II.
Key words: IGF-II, oxidative stress, parkinson disease, mitochondria

P159 Oxygen consumption, heart rate and sleep-wake cycle are impaired in Ts65Dn mice during early postnatal development

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Down syndrome (DS) is a genetic disease whose cause lies in the trisomy of human chromosome 21 and the overexpression of certain genes, being the main genetic cause of intellectual disability. In addition, DS affects various body systems including the neurological, musculoskeletal, respiratory, and cardiovascular systems, leading to complications that have a direct impact on people's life expectancy. Das et al (2013) demonstrated a high incidence of hypoxemia episodes in young and adult Ts65Dn mice, the most used animal model for DS. Ts65Dn adult mice have also been studied during sleep-wake cycle (Heinse et al., 2015). It has been demonstrated that this model present similarities to DS humans with respect to EEG alterations, presenting reduction of NoREM sleep, and an increase in wakefulness during light phase (Colas et al., 2008).
The aim of the present work has been to check how the sleep, respiratory and cardiac parameters are affected during postnatal development in Ts65Dn mutant mice.
For such purpose, breathing and heart rate, and the electrical activity at the neck muscles and brain of Ts65Dn mutant mice have been recorded during early postnatal development.
Our results demonstrate that Ts65Dn mutant mice exhibit a reduced respiratory rate in this period. Apnoeas appear also frequently during the first postnatal week. A similar reduction in heart rate has been observed. The sleep-wake cycle maturation is also affected and, as a result, on postnatal day 14 we observed modifications of time spent in NoREM sleep as well as in time spent in REM sleep.
Key words: Down syndrome, postnatal physiology, sleep-wake cycle.

P160 Functional interaction between dlPAG and nucleus Ambiguus: a neuronal unit recording study in rats.

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Introduction/objectives. We have previously examined the importance of the dlPAG region modulating laryngeal responses assessed via measurements of subglottic pressure in spontaneously breathing rats. Our studies demonstrated that the stimulation of the dlPAG decreased laryngeal resistance, modifying direct or indirectly the activity of laryngeal motoneurons of the nucleus Ambiguus (nA). The aim of our study is to confirm the possible electrophysiological interactions between the dlPAG and laryngeal motoneurons of the nA. To this end, we made extracellular recordings of putative nA neurons were obtained during dlPAG stimulation.
Methods: Experimental studies were carried out with non-inbred male rats (n=5), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/ kg, i.v., as necessary). Extracellular recordings of putative nA neurones were obtained during dlPAG stimulation (400 µs, 20-50 µA, 1 Hz).
Results: 160 putative neurons from the nA and its vicinity regions were registered. Depending on their typology we have divided them into 6 different groups: A. Inspiratory neurons with decreasing pattern of discharge (n=21); B. Inspiratory neurons with increasing pattern of discharge (=15); C. Expiratory E1 neurons. (n=15); D. Expiratory E2 neurons. (n=7); E. Cardiovascular neurons. (n=44); F. Neurons without cardiorespiratory modulation. (n= 54). A significant number of these recorded neurones, in all regions, modified their pattern of discharge from dlPAG stimulation (62%).
Conclusion: The results shown electrophysiological evidence of interactions between dlPAG and nA neurons. The possible mechanisms involved in these interactions are discussed.
Key words: dlPAG, nucleus Ambiguus, laryngeal motor neurons

P161 Morphological analysis of laryngeal outcomes from dorsolateral Periaqueductal Grey electrical stimulation in anesthetized rats: implications for c-Fos and FoxP2 expression within the three domains of the nucleus Ambiguus.

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Introduction/objectives: The dorsolateral Periaqueductal Gray Matter (dlPAG) controls fight/flight behavioural responses, triggering a defence response that is characterised by hypertension, tachycardia and tachypnoea. We have

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demonstrated the involvement of the dlPAG in altering laryngeal caliber. Furthermore, a heightened expression of the FoxP2 protein was described within the PAG. Our aim is to investigate the interactions between the dlPAG and neurons of the nA assessing the double immunofluorescence staining patterns of c-Fos/FOXP2 protein immunoreactivity (c-Fos-ir/FoxP2-ir) across the three primary subdivisions of the nA (compact, semicompact, and loose formation).

Methods: Experimental studies were carried out with non-inbred male rats (n=10), SPF, Sprague-Dawley (250-300 g) housed under standard conditions and anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/ kg, i.v., as necessary). The pattern of c-Fos-ir/FOXP2-ir and double immunofluorescence staining were examined throughout the nA region during electrical stimulation of the dlPAG (1 ms pulses, ranging from 30-50 μA, at 100 Hz, during 5 s). Guanethidine (10 mg/kg iv) was administered to suppress sympathetically mediated cardiovascular responses.

Results: dlPAG stimulation induced an increase in c-Fos-ir, with ipsilateral predominance in both the loose ($p<0.05$) and compact formation ($p<0.01$) within the nA. Furthermore, we could confirm the bilateral expression of FoxP2 across all nA domains. When analysing the co-expression of c-Fos-ir/FOXP2-ir within the loose formation of the nA, significant differences were observed ($p<0,001$) comparing stimulated and control animals.

Conclusion: Our study contributes with new data on the role of the mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Key words: dlPAG, nucleus Ambiguus, laryngeal motor neurons

P162 VIP $VPAC_1$ receptors as a target to prevent epileptogenesis and to attenuate cognitive decline in epilepsy.

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Introduction/Objetives: EEpileptogenesis is a major consequence of neuronal excitotoxicity, triggered by excessive activation of AMPA ionotropic glutamate receptors (Rs), that causes abnormal synaptic plasticity and neuronal excitability in the brain[1]. Vasoactive intestinal peptide (VIP) Rs are upregulated in patients with severe cognitive deficits due to Mesial Temporal Lobe Epilepsy (MTLE), thus suggesting an involvement of VIPergic mechanisms on abnormal synaptic plasticity phenomena, like long-term potentiation (LTP) (2,3). Since VIP role in epileptogenesis remains elusive (1), as its putative impact in the expression and function of AMPA Rs in epileptics, we set to evaluate the crosstalk between VIP R activation and $GluA_1$/$GluA_2$ AMPA R subunits in two "in vitro" models of hippocampal epilepsy.

Materials and methods: Hippocampal slices (400 μm) from male Wistar rats (n=3) were exposed for 10-min to hypoxia (HYPX, 5% CO2/95% N2) or oxygen-glucose deprivation (OGD, 5% CO2/95% N2 + partial replacement of glucose by sucrose) either in the absence or presence of the $VPAC_1$ R antagonist, PG97-269 (100 nM). Slices were collected 1h after for membrane preparation and Western blot analysis.

Results: Both HYPX and OGD, caused a major increase ($P<0.05$; n=3) in $VPAC_1$ R levels, without significant changes in VIP or $VPAC_2$ Rs. AMPA GluA1, but not GluA2, AMPA R subunits were enhanced ($P<0.05$; n=3) in hippocampal slices submitted to HYPX. Despite no changes in the GluA1 subunit levels were detected in OGD conditions, phosphorylation of the Ser831 residue of this subunit increased whereas Ser845 phosphorylation decreased. GluA1 subunit upregulation and enhanced Ser831 phosphorylation under HYPX and OGD conditions, respectively, was prevented with the selective $VPAC_1$ R antagonist, PG 97-269 (100 nM) suggesting an involvement of endogenous activation of $VPAC_1$ Rs.

Conclusion: Upregulation of $VPAC_1$ Rs in the hippocampus submitted to HYPX and OGD enhances AMPA R GluA1 subunit and/or its phosphorylation at the Ser831 residue, which fosters channel conductance and leads to enhanced synaptic plasticity. As such, $VPAC_1$ Rs may be a novel target to prevent epileptogenesis and decrease cognitive deficits in epileptics.

1. Cunha-Reis et al (2021) Pharmacol. Res. 164, 105356; 2. Serpa et al (2022) Neurochem. Int. 158, 105383; 3. Caulino-Rocha et al (2022) Biol. 11: 627.

Key words: VIP, VPAC1 receptor, Epileptogenesis, AMPA, GluA1, GluA2.

P163 Insight into changes of oxidative stress markers and Na,K-ATPase enzyme kinetics in the cerebellum of Zucker diabetic fatty rats based on diabetes severity

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Type 2 diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia contributing to insulin resistance, β-cell dysfunction, vascular complication, and chronic inflammation. Its pathogenesis is closely related to oxidative stress, which may affect brain functions and brain glucose metabolism due to its high metabolic activity and lipid-rich content. Na,K-ATPase is an essential enzyme maintaining intracellular homeostasis and energy balance, with properties that can sensitively mirror various pathophysiological conditions such as diabetes and neuroinflammation.

Following groups of male rats were used: Wistar (W, absolute control), Zucker Diabetic Fatty (ZDF) Lean (ZL, standard control), ZDF obese (ZO, hyperinsulinemia), and ZDF diabetic (ZOD, hyperglycemia). In addition to basic biometry and biochemistry, oxidative stress markers were assessed in plasma and cerebellar tissues. The cerebellar Na, K-ATPase enzyme activity was measured in varying concentrations of ATP substrate, and from that determined kinetic parameters - V_{max} and K_m.

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Our results showed significant differences in basic biometric and biochemical parameters within all studied groups. Furthermore, oxidative damage was greater in the cerebellum of diabetic rats compared with both controls. Interestingly, the Na,K-ATPase enzyme activity was the highest in the ZOD > ZO > W > ZL rats in a whole examined range of ATP.
In conclusion, an increase in systemic oxidative stress, resulting from diabetic conditions has a significant impact on the cerebellar tissue independently of diabetes severity. The more widespread development of diabetes due to disturbed Na,K-ATPase activity can also lead to various other pathologies, such as neurological ones. Supported by: APVV-21-0194.
Key words: Diabetes, cerebellum, oxidative stress

P164 Membrane progesterone and estrogen receptors regulate GABAergic transmission in the prefrontal cortex of male but not female mice.

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Introduction/Objetives: The prefrontal cortex (PFC) undergoes significant functional maturation of its GABAergic circuits during the transition from childhood to adolescence that is orchestrated by the sex hormones estradiol and progesterone. Disturbances in the hormonal environment during this period can lead to cognitive and affective disorders such as anxiety, depression, and schizophrenia. Little is known, however, about the sex hormone receptors involved in this functional reorganization and if there are sexual differences in this modulation. We hypothesize that sex hormones may modulate GABAergic transmission via the activation of membrane-bound receptors, e.g., membrane progesterone receptors (mPRs) and G-protein-coupled estrogen receptor (GPER), in the PFC.
Materials and methods: We first characterised the expression of mPRs and GPER in PFC by rtPCR and immunohistochemistry. We also studied the synaptic (phasic) and extrasynaptic (tonic) GABAergi transmission using *patch-clamp* recording in brains slices of female and male prepubertal mice.
Results: No differences were found in the total expression of mPRs and GPER or in the levels of phasic or tonic GABAergic transmission between males and females. Interestingly, selective activation of mPRs increased tonic GABAergic transmission in males but not in females while activation of the GPER increased phasic GABAergic transmission also only in males. We further demonstrate that this GABAergic modulation exerted by mPRs and GPER is dependent on protein kinases A and C, since their inhibition blocked this modulation.
Conclusion: This study sheds light on potential molecular targets (i.e. GPER and mPRs) to treat developmental disorders in the PFC.
Key words: Sex differences, GABAergic transmission, estrogen, progesterone, mPRs, GPER.

P165 Heart rate variability in Wistar rats under Zoletil anesthesia: exploring sex differences and light-dark dependence

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Introduction/Objectives: This study evaluates how the spectral powers of heart rate variability (HRV), namely very low frequency (VLF), low-frequency (LF), and high-frequency (HF), affect heart rate (HR), total spectral power of HRV, and the LF/HF ratio in Zoletil-anesthetized Wistar rats according to sex and light-dark (LD) period.
Materials and methods: Experiments were performed using spontaneously breathing Zoletil-anesthetized (i.p.; 30 mg/kg) Wistar rats (340 ± 40 g, 3-4 months of age, both sexes) after a four-week adaptation to an LD cycle (12h:12h). Animals were divided into four groups (n = 20/group) according to sex and LD period when the experiment was performed. Biosignals were analyzed using LabChart 8 (ID Instruments) 20 minutes after Zoletil administration from an average of 220 heart cycles.
Results: Zoletil induced tachycardia in both sexes and LD periods. Males also exhibited significant HR elevation during the dark period (415 ± 24 vs. 482 ± 19 beats/min, $p < 0.001$). The autonomic nervous system was involved in HR changes in both LD periods only in females. HR changes in males may be determined by other factors. In females, HRV correlated with sympathetic and baroreflex activity in both LD periods, while in males, with parasympathetic activity. LF significantly influenced the LF/HF ratio only in females, while the effect of HF on the LF/HF ratio was negligible in both sexes and LD periods.
Conclusion: Our work underlines the importance of considering sex and LD cycle in HRV studies.
Keywords: Heart rate variability, chronobiology, sex differences

P166 Sex-differential long-term cognitive consequences of alcohol and cannabinoid exposure during an adolescence-equivalent period in a rat model

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Introduction/Objetives: Prevalence data available on substance use in the Spanish adolescent population show that alcohol and cannabis are the most used substances, with a common dual consumption, specially in male population. Despite there is some controversy and few data available, this dual consumption during adolescent can induce long-term brain alteration with behavioural and cognitive consequences, increasing the risk of some mental disorder development. The aim of this study was to evaluate the long-term consequences of exposure to alcohol and cannabis during adolescent in male and female rats.
Materials and methods: Sprague-Dawley rats from both sexes were exposed to alcohol and Δ-9-tetrahidrocannabinol (THC, main psychoactive component from cannabis) during 1 month from around 35 post-natal day (considered an equivalent period to human adolescence). One group of animals (n=10 each) had 5% (v/v) of alcohol in drinking water

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for 24h every 3 days, another group was treated with THC (1,5 mg/kg, i.p., every 3 days), a third group received a combination of both treatments, and a control group of animals was similarly manipulated but without drug exposure. Cognitive and motor rat abilities were evaluated with different behavioural test just after drug exposure (during adolescence) and when they reached 10 months old: rota-rod test for motor performance, 8-arm radial maze for visual memory, and Barnes test for learning and memory evaluation.

Results: No significant short-term consequences were observed in any test result due to drug exposure. At the age of 10 months, male animals showed an impairment in both rota-rod and radial maze tests, that was not observed in female rats. Additionally, male animals exposed only to alcohol showed a tendency to worse performance in both tests, while females only showed this tendency in radial maze test. In Barnes test, no significant effect was observed in any group of treatment. Intriguingly, THC treated animals (both male and female) tend to find faster the scape box, however only males showed a clear worse execution in the reverse Barnes test.

Conclusion: There is a different behavioural long-term effect depending on sex after alcohol and THC adolescence exposure; as only male animals showed impaired cognitive

Key words: Cannabinoid, alcohol, adolescence, memory, motor skills

P167 Cilastatin as a potential anti-inflammatory and neuroprotective treatment in the management of glaucoma

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Introduction/Objetives: Glaucoma is a multifactorial neurodegenerative disease that causes blindness due to the loss of retinal ganglion cells (RGCs). We evaluated the protective role of cilastatin (CIL), an inhibitor of dehydropeptidase-1 (DHP-1) with anti-inflammatory properties in kidney failure, in an experimental glaucoma model.

Materials and methods: The model consisted of unilateral induction of ocular hypertension (OHT) by laser surgery, in the left eye (OHT eye) of male Swiss mice; the right contralateral (CONTRA) eye remained normotensive. Mice were daily administered with CIL (300mg/kg, i.p.), or its vehicle (VH, saline) starting 2 days before surgery and until sacrifice. The intraocular pressure (IOP) was measured. By immunohistochemical techniques, survival of RGCs and retinal inflammatory response, mediated by macroglial and microglial cells, were analyzed using Brn3a, GFAP and Iba-1, respectively. Furthermore, co-localization of inflammasome pathway markers: TLR4 and NLRP3, with RGCs, macroglia and microglia were characterized, together with DHP-1, via immunofluorescence, in OHT+VH and OHT+CIL mice.

Results: Surgery increased IOP in OHT eyes, but not in CONTRA. CIL did not affect IOP. OHT caused a higher RGCs loss in OHT than in CONTRA eyes; and CIL attenuated this RGCs loss in both eyes. Surgery increased macroglial and microglial activation in OHT eyes, and, to a lesser extent, in CONTRA eyes; CIL was able to prevent and/or revert the microglial and macroglial activation in both eyes. DHP-1 co-located with RGCs, microglia and macroglia; NLRP3 only co-located with RGCs and TLR4 exclusively with macroglial cells. DHP-1 expression diminished following CIL administration.

Conclusion: CIL treatment reduced the inflammatory glial response associated to glaucoma in this experimental model, and reduced the RGCs degeneration that causes blindness. We propose CIL to affect RGCs and glial cells by blocking DHP-1, thus further avoiding the internalization of detrimental inflammatory factors. Herein, CIL arises as a potential effective strategy to reduce OHT-associated retinal damage in mice.

Key words: Cilastatin; neuroinflammation; neuroprotection

P168 CoQUN as a potential strategy in the management of glaucoma in the visual pathway

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Introduction/Objetives: Glaucoma is a neurodegenerative disease that causes loss of vision, in which inflammation of the retina and several nuclei of the visual pathway have been observed. In this study, the potential therapeutic effects of the combination of Coenzyme Q10 and Citicoline (COQUN), with demonstrated anti-inflammatory properties, has been analysed in the visual pathway.

Materials and methods: An experimental model of glaucoma in Swiss male mice was used. It consisted of the unilateral induction of ocular hypertension (OHT) through the photocoagulation with laser of the left eye (OHT eye); while the right eye (CONTRA) remained normotensive. Mice were treated with the drug, COQUN, which was orally administered as a gelatine (500mg/kg citicoline, 200mg/kg CoQ10), or its vehicle (plain gelatine), during 15 days, before the surgery, and for 7 days after, until the sacrifice. Intraocular pressure (IOP) was measured, and the activation of microglial and macroglial cells analysed in the dorsolateral geniculate nucleus (dLGN) by immunohistochemical techniques using Iba-1 and GFAP, respectively, as markers.

Results: IOP was increased in the OHT eye 24 h after the surgery, and such an increment was attenuated after COQUN treatment; in the CONTRA eyes IOP did not change. Regarding microglia, although the total number of Iba-1+ cells remained unchanged, a more pronounced increment of its reactive morphotypes was observed in the right dLGN (contralateral to OHT eye) of the operated animals in comparison to the left dLGN (ipsilateral to OHT eye). The COQUN treatment partially reduced this microglial activation. Moreover, an increase in GFAP expression, i.e. macroglial activation, was observed in all the animals submitted to the surgery, in both the left and the right dLGN, whereas in the COQUN treated animals, a slight tendency towards a reduction of this activation was observed.

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Conclusion: Our data provide evidence for COQUN as a successful strategy to reduce glaucoma-associated glial activation in the dLGN. The evaluation of additional rely nucleus within the visual pathway is needed, together with the analysis of potential neuroprotective properties.
Key words: Glaucoma, visual pathway, microglia, macroglia, COQUN

P169 Cilastatin as a protectant against retinal damage in an animal model of malaria

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Introduction/Objetives: Malaria is a disease caused by *Plasmodium* parasites, that, despite curable, in some cases, progress to a severe form that can cause multi-organ failure, also affecting the Central Nervous System. Herein, in an experimental mouse model of malaria we have investigated the potential therapeutic efficacy of Cilastatin (CIL), an inhibitor of dehydropeptidase-1 (DHP), in the retinal damages induced by malaria in the retina.
Materials and methods: C57BL6 mice were infected by intraperitoneal inoculation with *Plasmodium berghei* ANKA infected erythrocytes (Malaria group, MAL). CIL (150mg/kg, i.p.) was daily administered for the following 5 days to both the infected (MAL) and the non-infected animals (Control, C). Parasitemia was then monitored every other day. Mice were sacrificed on day 6, blood samples collected, and retinas extracted for immunohistochemical analyses using Brn3a, GFAP and Iba1 markers to analyze retinal ganglion cells (RGCs), macroglial and microglia cells, respectively.
Results: MAL-mice showed a reduction in hemoglobin levels compared to C-mice; blood parameters were not affected by CIL. Retinas from MAL-mice showed a reduction in $Brn3a^+$ cells, but an increase in GFAP and Iba1 expression; MAL-mice also showed a change in microglial morphotypes towards activation and an increase in the number of vertical migratory morphotypes. CIL administration was able to prevent and/or revert all these effects.
Conclusion: The results provide evidence for CIL as an anti-inflammatory agent against the retinal damages observed in MAL infected mice.
Key words: Cilastatin, Malaria, Neurotoxicity, Neuroinflammation, Retina.

P170 Neuronal and glial alterations associated with treatment with the chemotherapy drug cisplatin: therapeutic approaches.

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Introduction/Objectives: Cisplatin (CIS) is a widely used antineoplastic agent employed in the treatment of many types of cancer. Renal toxicity is the main adverse effect of CIS administration, although neurotoxicity and visual alterations have also been described. Cilastatin (CIL), an inhibitor of dehydropeptidase-1 (DHP), has recently emerged as an anti-inflammatory drug. Therefore, we aimed to investigate, in male *Wistar* rats, the potential therapeutic efficacy of CIL in the CIS-induced adverse effects within the central nervous system.
Materials and methods: Rats were administered a single dose of CIS (5 mg/kg, i.p.) or its vehicle (VH, saline), and thereafter, for 5 consecutive days, CIL (150 mg/kg, i.p.) or its VH (saline). Rats were then sacrificed, and samples obtained. Retinas and brains were extracted and prepared for the immunohistochemical analysis in which neurons and glial cells were specifically identified by Brn3a or NeuN (neurons); GFAP (macroglia) and Iba1 (microglia) in the retina, dorsal lateral geniculate nucleus, superior colliculus (SC) and primary visual cortex (V1).
Results: CIS induced retinal damage by decreasing microglial and retinal ganglion cells expression, and subtly increasing GFAP expression. CIS significantly reduced NeuN expression only in V1, and induced an increase in Iba1 expression in SC. A generalized microglial activation was observed in all the visual relay nuclei studied. GFAP expression was not modified by these drugs. CIL was able to prevent and/or revert the CIS-induced microglial damage in the visual pathway but could not counteract the observed neuronal damage.
Conclusion: The chemotherapeutic agent CIS provokes a subtle despite remarkable neuronal and glial damage in the retina and the visual pathway; we have here demonstrated that CIL is able to prevent and/or attenuate the reported microglial activation expression, in all the studied areas, although CIL could not prevent the detected neuronal changes.
Key words: Cilastatin, Cisplatin, Inflammation, Neurotoxicity, Visual pathway.

P171 Responses of mouse inferior colliculus neurons to frequency-modulated tones

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Introduction: Mice commonly communicate using ultrasonic vocalizations in a variety of social contexts, and the characteristics of these vocalizations vary among mice strains. Many types of ultrasonic vocalizations consist of frequency-modulated tones. In our study, we were interested in the responses of neurons in the inferior colliculus (IC) of CBA mouse to frequency- modulated tones (4-80 kHz), which cover the frequency range of mice vocalizations.
Materials and methods: The responses of neurons to FM tones were recorded with a multichannel microelectrode in different parts of the IC in ketamine-xylazine anesthetized mice aged from 3 to 30 months.
Results: Responses to FM tones in the low frequency part of the central nucleus of the IC were characterized by a temporal pattern consisting of a sustained excitatory response, while in the high frequency part more complex responses consisting of several excitatory peaks separated by pauses were typical. In

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contrast, responses to FM tones in the external nucleus of the IC were relatively uniform regardless of position within the external nucleus. The complex temporal pattern of responses in the external nucleus consisted of multiple peaks. There were no significant age-related changes in responses of IC neurons to FM tones.
Conclusions: Our study suggests that inhibition may play an important role in the processing of species-specific vocalizations in subcortical parts of the auditory pathway in CBA mice and indicates what information about these complex acoustical signals IC neurons provide to higher parts of the auditory pathway.

P172 P11 as a feasible partner of the presynaptic machinery for neurotransmitter release at excitatory synapses of rat hypoglossal motoneurons

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Introduction/Objetives: The chaperone P11, also known as S100A10, regulates trafficking and membrane expression of several receptors and ion channels. We recently showed that P11 also controls neurotransmission at excitatory synapses of hypoglossal motoneurons (HMNs) (*IBRO Neurosci Rep* 2023;15(1):S345) by yet unknown presynaptic mechanisms.
Materials and methods: Physical interaction between P11 and MUNC13-1 was tested by co-immunoprecipitation and proximity ligation assays (PLA). Furthermore, adult and neonatal rats were injected with a small-interfering RNA against *p11* (siRNA$_{p11}$) into the fourth ventricle and brainstem slices of these animals were analyzed by confocal and electron microscopy.
Results: Co-immunoprecipitation and PLA supported the physical interaction and closeness (<40 nm) of P11 and MUNC13-1, a key protein of the presynaptic machinery for neurotransmitter release at excitatory synapses. Confocal microscopy analysis revealed a close relationship between P11, MUNC13-1, and the vesicular glutamate transporter-2 (vGLUT2) expressed in synaptic vesicles at excitatory synapses. Microinjection of siRNA$_{p11}$ resulted in a depletion of P11 and a decrease in MUNC13-1/vGLUT2 immunoreactive structures in the hypoglossal nucleus. Contrarily, electron microscopy analysis showed an increase in the linear density of excitatory (S-type) boutons attached to HMNs from siRNA$_{p11}$ -treated adult rats together with a reduction in active zone length and an increase in the size of the synaptic vesicle pool in these boutons.
Conclusion: P11 controls excitatory neurotransmission by interacting with MUNC13-1 and by regulating MUNC13-1 and vGLUT2 expression at synaptic terminals, then impacting on the presynaptic machinery of neurotransmitter release. Whether the apparently contradictory observations under electron microscopy after *p11* interference were the result of a compensatory response to the decrease in functionality observed at excitatory synapses merits further investigation.
Funding: MICIU (PID2019-110960GB-I00)
Key words: S100A10, MUNC13-1, vGLUT2

Renal physiology

P173 Antioxidant activity of myrtenal on renal ischemia/reperfusion injury

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Introduction/Objetives: Acute kidney injury (AKI) is a heterogeneous syndrome causing high mortality in intensive care units and vascular surgical operations. One of the major contributors to the development of AKI is oxidative stress. Myrtenal (Myrt), a potent antioxidant, is a monoterpene species found in many plants and fruits. This study aims to investigate the effect of Myrt pretreatment on AKI induced by ischemia-reperfusion (I/R) injury in rats.
Materials and methods: Forty male *Sprague Dawley* rats were randomly divided into four groups: Sham (i), I/R (ii), Myrt-40 (iii) and Myrt-80 (iv). Animals were treated with Myrt (40 and 80 mg/kg, intraperitoneally) or vehicle (Tween80, 0.05%) for 9 days before the procedure. Then, group i underwent laparotomy only. In groups ii-iv, bilateral renal pedicles were occluded with microvascular clamps for 45 min and then reperfused for 24 h. At the end of reperfusion, animals were euthanized and kidney tissues were obtained for biochemical analysis. Levels of MDA, SOD, GSH and CAT in kidney tissue were measured using spectrophotometric methods.
Results: I/R increased MDA levels in kidney tissue, whereas GSH, CAT and SOD levels decreased ($p<0.05$). Myrt pretreatment reversed this situation and increased GSH, SOD and CAT levels and decreased MDA levels in kidney tissue ($p<0.05$).
Conclusion: These findings indicate that Myrt exhibits a protective effect against renal I/R injury.
This research was supported by Inonu University Scientific Research Projects Unit (project no: TDK-2023-3271).
Key words: ischemia, reperfusion, myrtenal

P174 Na,K-ATPase kinetics and oxidative stress in kidneys of Zucker diabetic fatty rat

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Introduction/Objectives: Renal Na-K-ATPase represents one of the most important enzymes in homeostasis. Via Na^+ cotransport, it is responsible for glucose resorption in the proximal tubule. However, its function is altered differently in patients with diabetes mellitus type 1 and 2 (T2DM). In this study, we focused on investigation of renal Na,K-ATPase

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properties in the ZDF (Zucker Diabetic Fatty) rats as a model of T2DM, while monitoring parameters of oxidative status in renal tissue.
Materials and methods: 36 weeks old ZDF, Zucker lean (ZL) and control wistar (CW) rats were used. ZDFs were divided into 2 subgroups, as not all rats fully developed DM. The function of renal Na,K-ATPase was evaluated by kinetic measurements and together with markers of oxidative status were determined spectrofotometrically/spectrofluoremetrically
Results: The ZDF had more active renal Na,K-ATPase when compared to ZL, as well as CW, however, the enzyme in ZDF with DM had worsened ability to bind Na^+ when compared to CW. ZDF rats had increased TBARS and TAC in renal cortex. CW had lower GSH/GSSG in renal medulla than other groups.
Conclusion: In this study, renal Na,K-ATPase function among ZDF, ZL and CW rats were compared. Differences were noted in thiol–disulfide redox balance and Na,K-ATPase properties between ZL and CW rats, with ZL deviating towards ZDF values. ZDF rats exhibited higher lipid peroxidation, which was probably compensated by increased antioxidant capacity. Elevated renal Na,K-ATPase activities might be a consequence of increased glucose load in tubular fluid and higher demands for its reabsorption. Supported by grant APVV-21-0194
Key words: renal Na,K-ATPase, ZDF rats, oxidative stress

P175 Salivary Urea, Uric Acid, Creatinin Levels and Stress Axes in Chronic Kidney Disease

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Introduction/Objectives: In chronic kidney disease (CKD), uremic metabolites such as urea, uric acid and creatinine accumulate in the blood. There is not enough information about the reflection of these metabolites in body fluids such as saliva. In addition, the effects of these toxic metabolites on the autonomic nervous system (ANS) and hypothalamus-pituitary-adrenal axis (HPA), which are two important stress axes of the body, are not well known. The aim of the study was to observe whether these metabolites accumulated in the blood in CKD are reflected in saliva and to reveal to what extent they affect the stress axes.
Materials and methods: CKD patients (n=35) and healthy individuals (n=35) were included in the study. Blood and saliva samples were taken in the morning and urea, uric acid and creatinine levels were measured. Salivary cortisol measurement was performed to determine HPA activity. Electrocardiographical recordings (5 min) were taken to calculate heart rate variability (HRV) and hence, ANS activity.
Results: Blood urea, uric acid, creatinine levels and salivary urea and uric acid levels were found to be high in CKD ($p<0.05$). In CKD, salivary cortisol level was high and HRV was low ($p<0.05$).
Conclusion: In CKD, while all uremic metabolites increased in blood, only urea and uric acid increased in saliva. This finding suggests that uremia in CKD may be reflected in saliva. Additionally, the increase in cortisol and decrease in HRV in these patients indicate that stress is high. However, it is considered that stress may be due to increased psychological burden rather than uremia. This study is supported by the Scientific Research Unit of the Inonu University (No: 2022/2974)
Key words: Chronic kidney disease, uremia, stress

P176 Regulation of vascular tone of pre-glomerular vasculature by caldesmon

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Introduction/Objetives: This study explores the role of caldesmon (CaD) in age-related regulation of pre-glomerular vascular tone.
Materials and methods: Expression of CaD, myosin-light-chain (MLC_{20}), □-smooth-muscle-actin (□-SMA) and the targeting subunit of the myosin-light-chain-phosphatase, MYPT1 were assessed by western blotting in renal arteries (h-RA) from patients who underwent nephrectomy/enucleation, or murine interlobar arteries from young (~3 months; y-IA) and old (<22 months; o-IA) animals from wild type mice (WT), or mice carrying a global mutation for CaD (CaD+/-). Endothelial function, reactivity to □1-agonist, phenylephrine, activator of the soluble guanylate-cyclase, cinaciguat and inhibitor of RhoA-associated protein kinase (ROK), Y27632 were tested in IAs by wire-myography.
Results: In IA phenylephrine-induced contraction was unaffected by CaD-mutation or inhibition of endogenous NO-production by L-NAME. CaD-mutation did not affected acetylcholine-reactivity in all groups. Pretreatment with L-NAME had no effect on acetylcholine-relaxing response in y-IA and completely blunted it in o-IA from WTs. In contrast, in o-IA from CaD+/- this response was attenuated by only 50%. In all groups, CaD-mutation was without effect on cinaciguat-responsiveness, but led to rightward-shift of Y27632-relaxation. In IA from both ages mutation reduced CaD-expression by ~50%, while those of MLC20, □-SMA and MYPT1 remained unaffected. Consistent with the findings in mice, in h-RA CaD-expression correlated negatively with advanced age, while those of MLC20 and MYPT1 showed positive correlation.
Conclusion: This study provides evidence that CaD-expression determines endothelial reactivity and sensitivity to ROK of renal vasculature and seems to be a marker for pre-glomerular vascular aging.
Key words: Glomerulus, caldesmon-mutation

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P177 Role of cation channels in the pathophysiology of glycerol-induced acute kidney injury

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Introduction/Objetives: Acute kidney injury poses a significant public health concern, contributing to elevated rates of morbidity and mortality. In our study, we sought to explore the involvement of cation channels, known to influence renal physiology, in myoglobinuric acute kidney injury.

Materials and methods: "A total of 32 Sprague-Dawley rats were divided into four groups for our study. All rats underwent a 24-hour period of dehydration prior to experimentation. Subsequently, the control group received physiological saline, while the remaining groups (AKI24, AKI48, and AKI72) were administered intramuscular injections of 8 ml/kg of 50% glycerol solution. The control and AKI24 groups were euthanized 24 hours post-injection, the AKI48 group at 48 hours, and the AKI72 group at 72 hours. Urine, blood, and renal tissue samples were collected for the assessment of renal function and histological damage.

Results: Serum urea and creatinine levels exhibited a time-dependent increase, alongside reduced creatinine clearance. Moreover, kidney tissue analysis revealed elevated levels of TRPV1, TRPV4, and TRPC6 in the AKI72 group compared to the control. The AKI24 group displayed the most severe histological damage among the experimental groups.

Conclusion: It has been observed that renal function deteriorates over time in cases of myoglobinuric acute kidney injury (AKI). There is a growing belief that cation channels may play a role in this deterioration.

Key words: Cation channel, acute kidney injury, glycerol

Reproductive physiology

P178 Characterization of long-term alterations in a mouse model of prematurity induced by Mifepristone injection

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Introduction/Objetives: Individuals born preterm, particularly very preterm have increased risk of cardiometabolic diseases and alterations in neurodevelopment and behavior, both short and long-term. Animal models that mimic this condition are a useful tool to establish the mechanisms implicated, follow-up and evaluation of potential interventions. However, there is scarcity of studies on long-term consequences of prematurity in animals. Our aim was to establish a mouse model of preterm birth to study long-term cardiometabolic and neurological/behavioral outcomes.

Materials and methods: Pregnant CA1 female mice were mated and, after evaluation of conception (gestational day 0, G0), they were allocated to control (C, spontaneous delivery G20) or preterm (PT, induced delivery at G19 or G18). PT delivery was induced by s.c. injection of 0.2 mL of mifepristone (RU468; progesterone antagonist; 0.2 mL, 1 mg/mL, at 14:00 p.m) either at G18 (n=4 litters) or at G17 (n=1 litter). The offspring was followed until the age of 12 months, when blood pressure (tail-cuff plethysmography), fasting blood glucose, locomotor activity (open field), spatial cognition (Y maze) and anxiety-like behaviors (elevated plus maze; EPM) were evaluated.

Results: All litters were born on the night following Mifepristone injection (around 16h post-injection). G18-PT offspring (n= 1 litter) was born dead, while G19-PT offspring (n= 4 litters) survived until the age of 12 months with no differences in mortality rate or comorbidities compared to C (n=3 litters). At the age of 12 months PT male and female mice were normotensive, but male had significantly elevated glycemia compared to C (PT=142 ±□ 34.1 mg/dL; C=101 ±26.4 mg/dL; p=0.007) being unaltered in females (PT=101 ±25.8 mg/dL; C=101 ±22.1 mg/dL; p=0.98). No significant effects were observed in PT mice in EPM test. PT females showed a significantly lower percentage of alternations in the Y maze compared to C females (p=0.012), with no changes observed in males.

Conclusion: Mifepristone injection can be used to induce 1-day advanced delivery with long term survival in mice, which is associated with metabolic alterations in males and deficits in spatial memory in females in aged animals. Future studies will establish the spatial window of injection to induce earlier preterm delivery.

Key words: Glycemia, Blood pressure, Spatial Memory, Mifepristone, Preterm

P179 Elabela protects against ischemia/reperfusion injury and improves sperm parameters in a rat model of testicular torsión

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Objective: Ischemia/reperfusion injury following testicular torsion/detorsion can cause infertility. Potential protective effect of newly identified peptide Elabela on testicular injury was investigated.

Methods: Under ketamine-xylazine anesthesia, in male Sprague Dawley rats, 720o-clockwise rotated left testes (n=16) were untwisted after 2 hours. Elabela (40µg/kg) or saline was administered intraperitoneally twice, before and after detorsion. Sham-operated rats (n=8) received only saline. Testicular blood flow was measured using a Laser Doppler Flowmeter before and during torsion and after detorsion. After 4 hours, rats were euthanized via cardiac puncture. In testicular homogenates, lipid peroxidation (LP), antioxidant glutathione, myeloperoxidase activity (MPO), luminol, lucigenin and nitric oxide (NO) chemiluminescence levels were measured. Seminiferous tubule morphology was evalauted using Johnsen scoring on hematoxylin-eosin-stained sections. Epididymal sperm morphology and concentration were assessed. Statistical analyses were made with one-way ANOVA.

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Results: Testicular blood flow following detorsion was higher in Elabela-treated torsion-detorsion group than in saline-treated torsion-detorsion group ($p<0.05$). MPO, LP and glutathione levels were similar among groups. Elevated lucigenin, luminol and NO chemiluminescence levels in saline-treated torsion-detorsion group ($p<0.01$-0.001) were reduced in Elabela-treated rats ($p<0.01$-0.001). Johnsen scores and sperm concentrations were lower in saline-treated torsion-detorsion-group compared to those of sham-operated and Elabela-treated torsion-detorsion rats ($p<0.05$-0.001). Sperms with normal morphology were decreased in saline-treated, but not in Elabela-treated torsion-detorsion rats ($p<0.01$).
Conclusion: Elabela increased testicular blood flow, alleviated torsion-detorsion-induced oxidative injury and seminiferous tubule damage and improved sperm parameters, suggesting that further experimental and clinical studies are warranted to evaluate the therapeutic potential of Elabela in preventing testicular ischemia-induced infertility.
Key words: Testicular torsion, Elabela, Blood flow

P180 Functional impact of TRPCl/4/5 ion channels in the rat late pregnant and term myometrium

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Introduction/Objetives: Strong uterine contraction before term results in preterm labour, and failed contractions at term lead to caesarean delivery. Both scenarios leave the offspring with life-long health issues. Poor labour contractions also predispose the mother to postpartum haemorrhage. Clearly, there is an urgent need to improve our understanding of the mechanisms controlling uterine contractions during pregnancy and labour. TRPCl/4/5 ion channels are expressed in myometrium but their role in contractility has not been resolved.
Materials and methods: Membrane potential and contraction were recorded simultaneously in isolated strips of longitudinal myometrium from rats on day 18 ofpregnancy (not in labour, NIL) and during labour (IL, day 21).
Results: The TPRCl/4/5 selective antagonist picol45 (lOpM) depolarized NIL myometrium by 5.0±0.8 mV (n=7). This effect was reduced when calcium-dependent potassium channels were blocked by apamin and iberiotoxin. The response to pico145 IL was the disruption of sustained agonist-induced depolarization and transition from plateau-like to phasic contraction. Pico145 (30pM) enhanced oxytocin lOnM contraction (normalized area under the curve; from 76.6±7.5% to 115.4±12.6%; $p<0.05$) in NIL myometrium but was without effect on acetylcholine (ACh, 50μM).
Conclusion: Our observations support a role for TRPCl/4/5 in regulating uterine contractions in rat longitudinal myometrium during pregnancy. Before labour calcium influx via TRPCl/4/5 activates calcium-dependent potassium channels, contributing to the required quiescence. This effect is not observed IL, permitting myometrium contraction.
Key words: Pregnancy, myometrium, TRPCl/4/5 ion channels

P181 The role of ferroptosis in high fructose diet-induced lung injury

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Introduction/Objetives: Despite intensive research, lung injury remains a significant public health problem due to its high mortality and morbidity and limited treatment options. Ferroptosis is a new form of programmed cell death. In this study, we tried to investigate the role of ferroptosis in high fructose diet-induced lung damage.
Materials and methods: A total of 34 male Sprague-Dawley rats were used in the study in 4 groups. The control group received tap water and the other groups received tap water containing 20% fructose. The control group was followed for 1 week, the F4 group for 4 weeks, the F8 group for 8 weeks, and the F12 group for 12 weeks. Glutathione, malondialdehyde, glutathione peroxidase 4, acyl-CoA synthetase long chain family member 4 (ACSL4), transferrin receptor 1(TFR-1), tissue iron levels and histopathologic changes were examined in lung tissues.
Results: In the group fed high fructose for 12 weeks, ACSL4, TFR1 and tissue iron levels were higher than in the control group. High lung injury was observed in the high fructose group compared to the control group. Fructose-rich diet had detrimental effects on lung histology, and these effects increased over time.
Conclusion: Our study conjectured that ferroptosis could potentially underlie the detrimental impact of elevated fructose intake. Nevertheless, to ascertain conclusive findings, additional studies incorporating a broader spectrum of fructose dosages and durations of study are imperative.
Key words: High fructose diet, lung injury, ferroptosis

P182 Biophysical stability of surfactant preparations in the presence of various inhibitors

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Introduction/Objetives: Pulmonary surfactant is a complex mixture of lipids and proteins that reduces the surface tension on the surface of the alveoli, thus significantly contributing to their stabilization at the end of expiration. Different inhibitors, including plasma proteins can interfere with lung surfactant. We aimed to evaluate the biophysical activity of exogenous surfactants after interaction with potential inhibitors that can reach the lungs by inhalation or transfer from the blood.
Materials and methods: Evaluation of surface activity of different surfactants was evaluated with a pulsating bubble surfactometer. Surfactants (animal derived: Poractant alfa, synthetic: CHF5633, synthetic with recombinant peptide: Combo) were diluted to 1-2.75mg/ml of phospholipids. Dose related effects of surfactants on minimum surface tension (ST) under bubble pulsation alone, or in the presence of HCl, albumin or fibrinogen were investigated.

Results: After 5 minutes of pulsation, minimum ST of Combo and Poractant alfa at a concentration of 2mg/ml was significantly lower in comparison with CHF5633 at 2mg/ml ($p<0.05$ to 0.01). Mixture of surfactants and HCl (2.5mgPL/ml, pH1.25) had a significant effect on the minimum ST on Poractant alfa and Combo ($p<0.05$ to 0.01). Addition of albumin or fibrinogen significantly increases minimum ST of Poractant alfa and CHF5633 ($p<0.05$ to 0.01). In addition, Poractant alfa and CHF5633 with albumin or fibrinogen report significantly higher minimum ST compared to Combo ($p<0.05$ to 0.001).
Conclusion: Surfactants interfere differently with HCl and plasma proteins. Differences in surfactants composition and/or the different production could affect their biophysical activity.
Acknowledgment: VEGA 1/0004/21, VEGA 1/0097/23
Key words: Exogenous surfactant, surface tension, inhibitor

P183 Effect of roflumilast and dexamethasone on pulmonary function and oxidative changes in meconium-induced lung injury

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Introduction/Objetives: Meconium aspiration syndrome (MAS) is a serious neonatal condition with a complex mechanism of action including oxidative and inflammatory processes, surfactant dysfunction, development of pulmonary oedema and respiratory failure. Exogenous surfactant delivery and ventilation support are the fundamentals of MAS treatment. In addition, meconium-induced oxidative and inflammatory activities in lung may render the therapeutic surfactant inactive. Our goal was to determine whether, in an animal model of MAS, anti-inflammatory medication (corticosteroid dexamethasone or selective phosphodiesterase inhibitor roflumilast) improves lung function and oxidative damage.
Materials and methods: EWhen meconium-induced respiratory failure ($P/F<10$ kPa) occurred in New Zealand rabbits, intravenous boluses of either roflumilast (ROFL; 1 mg/kg) or dexamethasone (DEX; 0.5 mg/ml) were administered, or no therapy (MAS). Before and 30 minutes following the delivery of meconium, as well as 30 minutes, 1, 2, 3, and 4 hours following treatment, blood gases and ventilatory parameters (P/F, OI, PaCO2) were recorded. In bronchoalveolar lavage fluid (BALF), pulmonary oedema, oxidative damage (TBARS, AOPP, TAC), and signalling molecules (MCP-1, ET-1, H1F) were determined.
Results: AAnti-inflammatory therapy increased OI ($p<0.05$ DEX vs. MAS) and P/F ratio ($p<0.05$ ROFL vs. MAS) as early as 1 hour after dose. ROFL and DEX both significantly reduced TBARS ($p<0.05$), ET-1 ($p<0.01$), MCP-1 ($p<0.05$), and H1F ($p<0.05$) in comparison to the untreated group, as well as both AOPP therapies ($p<0.05$ vs. MAS).
Conclusion: In experimental MAS, anti-inflammatory treatment with dexamethasone or roflumilast improved ventilatory parameters and oxidative stress. The concentrations of signalling molecules in BALF were as well impacted by dexamethasone.
Acknowledgment: VEGA 1/0004/21, VEGA 1/0097/23
Key words: Meconium aspiration syndrome, lung damage, pulmonary function

Teaching of physiology

P185 Service-learning educational approach for undergraduate students: development of an outreach workshop for high school students

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Introduction/Objetives: In the last decade, universities have strengthened the development of outreach activities, involving professors, researchers and graduate students but with little or no implication of undergraduate students. However, the development of this type of activities, using the Service-Learning educational approach, could be a valuable tool for the acquisition of learning competencies by undergraduate students of Health Science Degrees and would put bioscience at the service of society.
Materials and methods: we developed a workshop entitled "Exploring the human body", in which 205 students of either Nursing or Medicine Degrees (University of Málaga, Spain) acted as mentors of 753 high school students (15-16 years old) since 2016 to 2023 (excluding 2020 and 2021 due to the COVID-19 pandemic). The workshop consisted of five work stations. Each of them featured a set of different experiments and activities, designed to teach physiological and biomedical concepts related biomolecules, cells, tissues, organs and systems. Both high school and undergraduate students gave an evaluation of the workshop via questionnaires (Likert scale-based and short-answer questions).
Results: Data showed an overall score of 4.6 out of 5 points for the workshop by both high school and undergraduate students. Undergraduate students pointed out that their participation had a positive impact on their academic background (4.8 out of 5 points), mainly due to the improvement of their oral communication skills and self-confidence.
Conclusion: These results suggest that this methodology would be valid and applicable to develop the transversal competences of the students in Bachelor's degrees of Health Sciences.
Key words: Service-learning, outreach, undergraduate

P186 Integrated laboratory classes to learn physiology in a psychology degree: impact on student learning and experience

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Introduction/Objetives: Physiology content is perceived by students as rather difficult, who may lack vision on how to relate it with their professional training. Therefore, identifying novel active and more engaging pedagogical strategies for teaching physiology to psychology students may help to fill this gap. In this pilot study, we used the PBL methodology developed around a clinical case to evaluate psychology students' experience and learning in two laboratory classes modalities. The aim of this study was to compare undergraduates' preference for laboratory classes taught either independently (cohort 1, n = 87 students) or integrated into the PBL-oriented clinical case (cohort 2, n = 92 students) for which laboratory classes were transformed into Integrated Laboratory Classes (ILCs). The students' academic performance was also evaluated to look for quantitative differences between cohorts

Materials and methods: In this pilot study, we aimed to assess the impact of academic performance and student's experience of teaching laboratory classes contextualized within the PBL applied to Physiology teaching in first year psychology degree. Two cohorts from consecutive academic years were selected: cohort 1 (n = 87) from 2018/19, which was considered the control group, and cohort (n = 92) during 2019/2020 as the experimental group. Cohort 1 was organized in three homogeneous group of students each of 25–30 students. Cohort 2 was organized in four heterogeneous group of students, two of them composed by 30 students and the other two of lessthan 30 students. For quantitative analysis, academic scores from each of the different blocks in both cohorts were compared using an inferentialnon-parametric statistical study. Additonaly a questionnaire to assess student´s experience was distributed.

Results: When we compared the academic scores obtained in the theoretical content from each cohort, we found a significant improvement ($p < 0.05$) in cohort 2 where the students achieved better results as compared to cohort 1. A subset of students was asked to fill a questionnaire assessment on their experience and found that 78.9% of them preferred integrated laboratory classes over laboratory classes alone. They consistently reported a better understandingof the theoretical content and the value they gave to ILCs for learning.

Conclusion: In conclusion, our pilot study suggests that integrating laboratory classes into PBL-oriented clinical contexts help to retain core physiology contents and it can be considered as an engaging learning activity worth implementing in Psychology teaching.

Key words: integrated learning, PBL methodology, laboratory classes.

P187 Implementation of problem-based learning (PLB) methodology for neurophysiology teaching in speech therapy degree

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Introduction/Objetives: Problem-based learning (PBL) is a student-centered teaching method in which students learn about a subject solving an open-ended problem, based on analysis, resolution and discussion in groups. The aim of this study is to obtain a descriptive analysis of the implementation of the PBL methodology in the course Neurophysiological Bases of Behaviour, in which fourth-year undergraduate students of Speech Therapy Degree were enrolled in the academic years 2020-21, 2021-22 and 2022-23 at the University of Malaga.

Materials and methods: In the study, 139 undergraduate students were included. They answered two surveys, which included the same items (Likert scale questions and personal opinion question), at both the beginning (to know students' expectations) and the end (to measure the level of satisfaction) of the course. The surveys allowed, on one hand, to analyse the students' assessment on different issues related to the technical and methodological aspects of PBL, their participation, group work and personal skills acquisition. On the other hand, we studied whether the students contemplated significant differences between PLB methodology and other traditional teaching methods, regarding the development of abilities and competencies.

Results: The results of the initial survey showed a very positive attitude of the students towards the implementation of PBL methodology in all the academic years. Compared to this, in the final survey, the scores obtained in the items regarding the skills and competencies acquired were similar or significantly higher.

Conclusion: It is worth highlighting the higher effectiveness of PBL for students to acquire competences, so this methodology could be applicable in other degrees of Health Sciences and general concepts of physiology.

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Key words: Problem-based learning, neurophysiology, undergraduate

P188 Educational Intervention in Medical Physiology: Case-Based Learning with Residents

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Introduction/Objetives: During the academic year 23-24, we implemented an educational intervention in the Physiology I and II courses for second-year medical students. The intervention aimed to enhance learning by integrating case studies in each thematic block covering general physiology, cardiovascular system, respiratory system, endocrine system, renal system, and digestive system. The intervention was designed to integrate theoretical knowledge with practical clinical scenarios.

Materials and methods: Case scenarios were developed by residents of various specialties who had graduated from our university, in collaboration with the physiology faculty. Cases, including data from history, physical examination, and ancillary tests, were presented to students over a two-week period with subsequent guided questions. Students were required to submit their conclusions about the case. Finally, a collaborative clinical session led by the resident physicians was organized. These sessions provided an opportunity for students to interact with residents, discussing both case resolution and personal experiences, including preparation for the MIR exam and specialization.

Results: Surveys were conducted to measure perceptions, and concept assimilation will be evaluated through specific test questions.
Conclusion: The sessions proved to be mutually enriching, providing students with insight into their future profession while providing residents with teaching experience and reinforcing the importance of core subjects.
Key words: Case-based learning, medical education, collaborative teaching, student-physician interaction.

P189 Hybrid education in physiology learning

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The complexity in learning physiology lies in understanding the regulation mechanisms of physiological processes and the diversity of interconnections between the different systems that make up the organism. Typically, students do not have excessive difficulties in learning the cellular and molecular mechanisms that govern specific processes (for example, the cardiac cycle) but encounter problems when it comes to learning the different forms of adaptation to internal or external changes to the organism, as well as the ultimate purpose of these processes (following the example, chronotropic and ionotropic cardiac regulation for the maintenance of adequate cardiac output). The traditional lecture format is insufficient if we aim for students to reach higher cognitive levels of learning, such as analysis, application, or evaluation of physiological parameters that enable the learning of this subject in all its complexity. Therefore, in recent years, we have developed a hybrid education method, combining elements of face-to-face teaching and online teaching, for learning physiology in the biomedicine degree (Francisco de Vitoria University).
We design the course by intertwining the use of materials for asynchronous study with cooperative activities and participative presentations for face-to-face time. The asynchronous materials consist of videos created with a lightboard by the teacher herself, as well as various self-regulated learning tasks, all shared through the virtual platform and the subject's own social media networks (youtube, instagram, and telegram).
The evaluation of this model by students is very high, and the academic results are quite good. Currently, we are analyzing the impact on deep learning.
Key words: Hybrid education, asynchronous study, cooperative activities, media networks

P190 From systems to homeostatic variables: a new organizational proposal for teaching physiology

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Despite being a science that studies function rather than structure, all physiology courses and textbooks are organized by anatomical systems. While it is true that the very definition of a system refers to function, teaching physiology with the same organization as anatomy might hinder achieving a deep understanding of the threshold concept of this science: the maintenance of homeostasis of the internal environment. This anatomically fragmented teaching facilitates a mechanistic understanding of physiology but makes it difficult to 1) recognize the integrative reality of the living organism under study, even though the relationship between systems is the inherent difficulty of the science itself; and 2) comprehend the teleological aspect of the science, meaning that learning mechanisms and processes is prioritized over understanding functions in terms of purpose and end. Regarding the latter, we do not propose to regress in terms of studying and understanding physiological mechanisms but to redefine their purposes, focusing on the variables of the internal environment and their homeostasis. For example, our students must understand the mechanisms of renal or cardiovascular functions, with a clear vision that both converge in maintaining a vital variable of the internal environment: blood pressure. With these premises, we have made a change in the thematic organization of the physiology course, moving from an anatomical organization to a more integrative one focused on 1) the elements of feedback systems, 2) the differentiation of bodily functions into motor, visceral, and cognitive, and 3) the variables of the internal environment on which the maintenance of life depends.
Key words: Teaching physiology, Threshold concepts, teleological aspects.

P191 Virtual laboratories (VL) complement but should not replace face-to-face practical classes: Evaluation of undergraduate perceptions of VL resources for practical skill learning in life sciences

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Introduction/Objectives: Virtual laboratories (VLs) enable students to experiment, analyze data or interact with digital content in a non-physical space. VLs include simulations, electronic notebooks, videos and augmented reality. As part of the 'VL Project', comprising 5 academic institutions in Ireland, we sought to determine how VLs could enhance practical learning in undergraduate life science students at Dundalk Institute of Technology (DkIT).
Materials and Methods: From 2021-2024, we exposed students to VLs across 7 science degrees (e.g. BSc Bioscience, BSc Pharmaceutical Science, BSc Biopharmaceutical Science). We focused on Labster simulation software, and Lab Archives electronic notebooks. Over 600 students performed VLs in 14 modules from 1st-4th year (e.g. Biotechnology, Immunology, Bioanalytical Science). We surveyed student perceptions both before, and after using VLs and conducted focus groups to evaluate emergent themes in depth.
Results and Conclusion: Among respondents (n=263), the most beneficial component of laboratory experiences, as indicated by 58% of students was experimental work (opposed to pre-practical talks or post-laboratory assessments). Additionally, 90% of students agreed with the statement: "VLs

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enhanced my level of confidence with experimental science". Moreover, 75% of students stated VLs should only be used to complement face-to-face (F-2-F) teaching. Thematic focus group analysis revealed students valued VLs as flexible pre-lab tools, allowing repeated engagement with, and troubleshooting of experiments in a safe, non-time limited manner. However, students reinforced they valued hands-on experience, in-person instructor guidance and real-world demonstration over VLs for experimental work. VLs can complement but should not replace F-2-F laboratory experiences in undergraduate life sciences.

Key words: Practical skills, virtual laboratories, digital learning

P192 Unlocking the potential of video-based lessons to improve the learning experience in Physiology

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Introduction and objectives: Human physiology is a crucial subject in health science degrees, and at the University of Valencia (UV), we teach it through lectures, practical classes, and tutorials. Practical classes have limited resources, making video learning a valuable alternative, as we retain 50% of what we see and hear, according to Glasser's pyramid. Therefore, we have created a series of videos to bridge the gap between lectures and practical classes.

Materials and methods: Two videos (5 minutes long) were created to explain simple and forced spirometry to students of Human Physiology in the Physiotherapy (n=115) and Medicine (n=30) degrees at UV. We instructed the students to watch the videos before the practical sessions. We surveyed students to assess the impact of videos on their self-learning. Two questions were asked: 1) Can you perform or interpret a spirometry test after watching the videos? 2) Rate the material for self-learning on a scale of 1-10.

Results and conclusion: After watching the videos, 16.5% of physiotherapy and 30% of medical students reported being able to perform and interpret spirometry perfectly, while 81.7% of physiotherapy and 63.3% of medical students claimed to believe they could at least try. Physiotherapy and medical students found the material useful for learning, with scores of 8.8 and 8.0, respectively. The videos are available on-demand, making them perfect for asynchronous learning. We recommend watching the videos before practical sessions. In conclusion, the use of video-based lessons that are tailored to the individual needs of students can improve self-learning.

Keywords: Teaching, physiology, video-based lessons

P193 Evaluating physiology within the European Higher Education Area: insights from Portuguese universities and colleges

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Introduction/Objetives: Discussions regarding the evolving landscape of Physiology education and research within the European Higher Education Area (EHEA) is recurrent, especially after more than twenty years of the Bologna process. This study shows how Physiology is currently taught in Portugal.

Materials and methods: This study uses a two-stage mixed-methods approach to evaluate institutions with Physiology courses and faculty research. Data was collected from the Portuguese government agency DGES from September to November 2022. Information on class type, hours, and ECTS was gathered from institutional websites, creating a database of 365 courses and 764 distinct Physiology curricular units. The second phase involved a bibliometric analysis of faculty research from 2017 to 2022, using VOSviewer for network analysis to identify key research themes.

Results: Significant variations were observed between universities and technical colleges. Physiology is often a foundational subject in the first year at technical colleges, whereas at universities, it is integrated throughout the curriculum, reflecting a deeper engagement with advanced knowledge. The study's bibliometric analysis highlighted a contrast in research output between universities and technical colleges, with faculty at leading universities more prolific. Key research areas included biomedical innovation, cardiovascular health, sports performance, and wellness.

Conclusion: These findings, while specific to Portugal and the EHEA, underscore the critical role of Physiology in health education, reflecting a still insufficient characterization of the physiology teaching systems and physiologists' community within the EHEA. Despite disparities, it is clear that physiology is a vital part of life sciences and is essential for addressing health and disease issues.

Key words: Physiology Education; University vs. Technical College, Bibliometric Analysis

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