



Abstracts of the XXXIX Congress of the Spanish Society of Physiological Sciences (SECF), 18-21 September 2018, Cádiz, Spain

Dear colleagues,

At the last Congress, the Society entrusted to the Organizing Committee the honor to host the XXXIX Congress of the Spanish Society of Physiological Sciences (SECF) in the ancient city of Cadiz. More than half a century later, Cadiz will return to host this important Congress from 18th to 21st September 2018.

We responsibly accept the task with the difficult objective of maintaining the high scientific standards reached in previous meetings and, to fulfil the SECF-Congress philosophy, that is, “to generate interest, stimulate research and disseminate knowledge of Physiology and its applications”. We hope that this meeting provides, not only, a framework in which we have the opportunity to learn about current research lines of our colleagues in the field of Physiology and Pathophysiology, but also an environment in which to wake up the interest of the newly graduates in research.

Studies originated from Spanish and worldwide laboratories have been carefully selected by the international Scientific Committee to assure scientific excellence of the event. These studies will be presented in the form of plenary lectures, symposia, oral communications and posters including, for the first time, a special session for End-of-Degree projects of contrasted quality. Topics cover a wide range of subjects, among them

Cell and Molecular Physiology, Neurophysiology, Neurodegeneration, Neurogenesis, Endocrinology, Metabolism, Nutrition, Obesity, Cardiovascular Physiology, Blood, Respiratory Physiology, Gastrointestinal Physiology, Renal Physiology, Reproductive Physiology, Chronobiology, Animal Experimentation, Teaching of Physiology, Sports Physiology, Optogenetic and Mitochondrial Physiology.

We would like to express our sincere acknowledgment to the contributing authors, especially to the invited speakers, some of them coming from the opposite part of the globe, chairpersons and colleagues from de Local, Organizing and Scientific committees, who have worked selflessly to get a high quality Congress from the scientific and social points of view. We greatly appreciate the indispensable economic support provided by commercial and institutional sponsors who have made this meeting possible.

We are honored to welcome all participants, wishing you a scientifically rewarding, personally enriching and productive Congress. We hope you enjoy the hospitality of Cadiz, its wide range of cultural and gastronomic offer, its weather and, of course, its incomparable beaches.

Bernardo Moreno-López
President of the Organizing Committee

XXXIX Congress of the Spanish Society for Physiological Sciences (SECF)

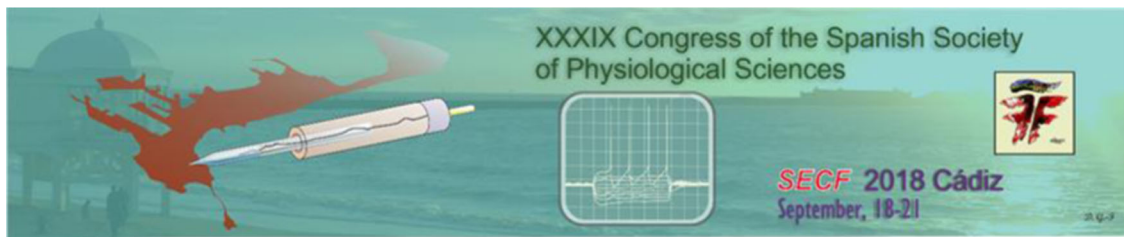
Cádiz, September 18–21, 2018

Tuesday, September 18, 2018		
Hour/ Room	Bolívar	Argüelles
15:30	Registration Office Opens	
16:00	Symposium 1 Title: <i>Adult Stem Cells</i>	Oral Session 1 <i>Cell & Molecular</i>
16:30		
17:00		
17:30		
18:00	Break	
Room	Aula Magna	
18:30	Opening Lecture/Plenary Lecture 1	
19:00	<i>Exercise is Medicine: Efficacy, dosing, and side effects.</i>	
19:30	Dr. Carl J. Lavie	
Welcome reception		

Wednesday, September 19, 2018			
Hour/ Room	Lequerica	Argüelles	
9:00	Symposium 2 Title: <i>Organoids</i>	Oral Session 2 <i>Sports & Pregnancy</i>	
9:30			
10:00			
10:30			
11:00	Coffee Break/exhibitions		
11:30	Poster session 1: <i>Cell & Molecular, Sports, Teaching, Neurophysiology</i>		
Room	Bolívar	Argüelles	
12:00	Symposium 3 Title: <i>Innovation in Physiology Teaching</i>	Oral Session 3	
12:30			
13:00			
13:30			
14:00	Lunch Break		
14:30	Lunch Break		
15:00	Book Launch		
15:30	Technical Workshop		
Room	Argüelles	Bolívar	Lequerica
16:00	Symposium 4 Title: <i>Immunomodulatory and antioxidant properties of melatonin</i>	Symposium 5 Title: <i>Exercise for the prevention and treatment of cardiovascular disease</i>	Oral Session 4 <i>Neurophysiology & Optogenetic</i>
16:30			
17:00			
17:30			
18:00	Break		
Room	Aula Magna		
18:30	SECF Annual Meeting		
19:00	Antonio Gallego Award		
19:30	Dr. Ginés Salido Ruíz		
Social Program (optional)			

Thursday, September 20, 2018			
Hour/Room	Lequerica	Bolívar	Argüelles
9:00	Symposium 6 Title: <i>Current guidelines and new trends in animal research</i>	Symposium 7 Title: <i>Cardiovascular Risk: from basic physiology to clinical research</i>	Oral Session 5 <i>Endocrinology, Metabolism & Nutrition</i>
9:30			
10:00			
10:30			
11:00	Coffee Break/exhibitions Poster session 2: <i>Cardiovascular & Respiratory, Chronobiology, Endocrinology, Metabolism & Nutrition, Animal Experimentation, End-of-Degree Projects</i>		
11:30			
Room	Aula Magna		
12:30	Plenary Lecture 2		
13:30	<i>Setting the biological clock and the importance of non-visual light detection in fish.</i> Dr. David Whitmore.		
13:30			
14:00	Lunch Break		
14:30			
Room	Bolívar		Argüelles
15:00	Technical Workshop		Book Launch
15:30			
Room	Argüelles	Bolívar	Lequerica
16:00	Symposium 8 Title: <i>Obesity and adipose tissue. New perspectives</i>	Symposium 9 Title: <i>Recent advances in chronobiology</i>	Oral Session 6 <i>Cardiovascular II</i>
16:30			
17:00			
17:30			
18:00	Break		
Room	Bolívar		Argüelles
18:00	Symposium 10 Title: <i>Telomeres, Diet and Human Disease</i>		Oral Session 7 <i>End-of-Degree Projects</i>
19:00			
Social Program (optional): Conference Dinner			

Friday, September 21, 2018			
Hour/Room	Argüelles	Bolívar	Lequerica
9:00	Symposium 11 Title: <i>Mitochondrial physiology, reinventing the complexity</i>	Oral Session 8 <i>Neurodegeneration</i>	Oral Session 9 <i>Gastrointestinal</i>
9:30			
10:00			
10:30	Coffee Break/exhibitions Poster session 3: <i>Neurodegeneration & Neurogenesis, Clinical & Traslational, Gastrointestinal, Renal & Epithelial, Reproductive, Blood</i>		
11:00			
Room	Bolívar		Argüelles
11:30	Symposium 12 Title: <i>Brain Stimulation</i>		Oral Session 10 <i>SEFC Awards & Teaching</i>
12:00			
12:30			
Room	Aula Magna		
13:00	Closing ceremony		
13:30	SECF's 2018-Juan Negrín Award Lecture		
14:00	Dr. Fernando Cerveró Santiago		
14:30	<i>Grey matter under grey skies: memorias de un fisiólogo itinerante</i>		
Social Program (optional)			



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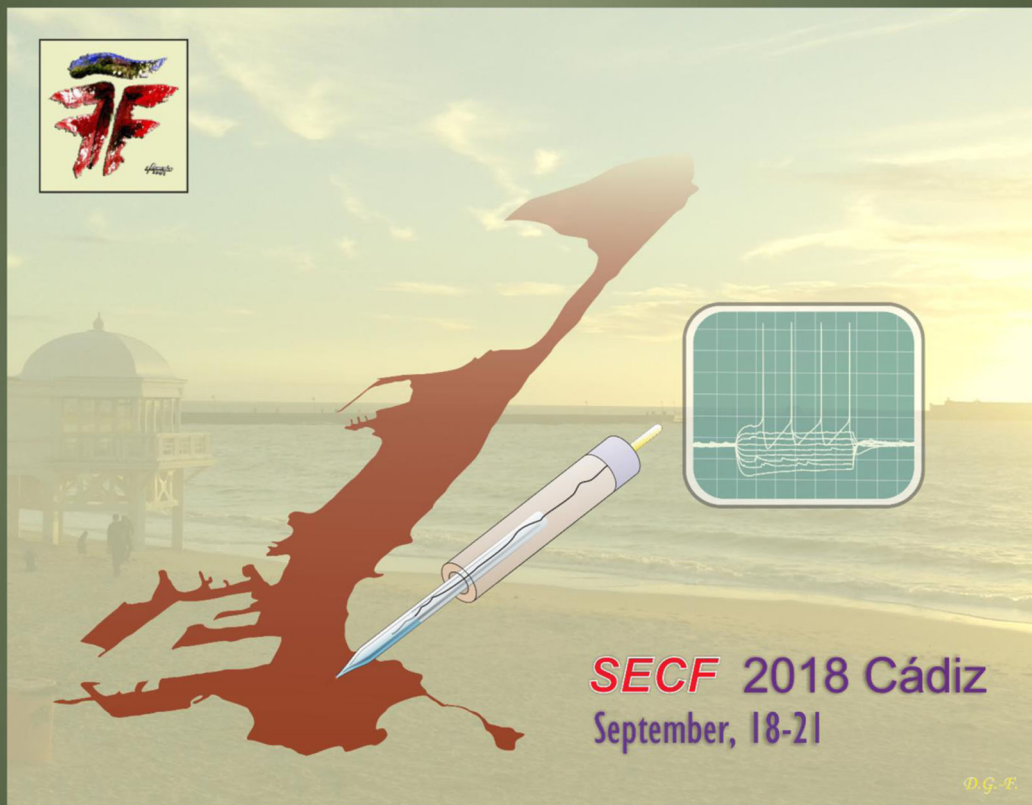
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ABSTRACT BOOK

XXXIX Congress of the Spanish Society of Physiological Sciences



Edition

Castro González, Carmen
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LECTURES

L1 (Opening Lecture / Plenary Lecture 1)

STATUS OF CARDIAC REHABILITATION AND EXERCISE TRAINING IN SECONDARY CARDIOVASCULAR PREVENTIONC. J. Lavie^(1,2)⁽¹⁾ John Ochsner Heart and Vascular Institute, Louisiana, USA; ⁽²⁾ Ochsner Clinical School, University of Queensland, Brisbane, Australia

Coronary heart disease (CHD) remains the leading cause of morbidity and mortality in the United States and in most of the rest of the world. In patients with CHD events and with stable systolic heart failure (HF), there is substantial evidence to indicate high risks of subsequent major cardiovascular disease (CVD) events and CVD-mortality. Considerable data, including from my colleagues and I, has indicated the benefits of formal cardiac rehabilitation and exercise training (CRET) programs to improve CHD risk factors, especially psychological risk factors and levels of cardiorespiratory fitness (CRF), in the secondary prevention of CVD. Although many factors improve with CRET programs, which will be reviewed, most of the emphasis has been directed at improvements in CRF, as these seem to particularly correlate with improvements in psychological risk factors, stress-related increased mortality, as well as all-cause mortality. In my lecture, although I will review historical data regarding the benefits of formal CRET programs in secondary prevention of CVD, I will mostly emphasize the data obtained from the studies that my colleagues and I have published from the Ochsner Clinical School on the benefits of CRET on coronary risk factors, including metabolic syndrome, inflammation, psychological risk factors (anxiety, hostility, but mostly depression), CRF, and all-cause mortality. Greater efforts are needed to promote formal exercise training, specifically CRET, in the secondary prevention of CVD, especially CHD.

L2 (Plenary Lecture 2)

SETTING THE BIOLOGICAL CLOCK AND THE IMPORTANCE OF NON-VISUAL LIGHT DETECTION IN FISH

D. Whitmore

Dept. of Cell and Developmental Biology, University College London, London, UK

It is well-established that fish tissues and cells contain an endogenous circadian clock that regulates the daily timing of key events within each cell. The more surprising aspect of fish biology is that the majority, if not all, cells in the body appear to be directly light responsive. As a consequence, these cells and tissues must contain the photopigments/opsins necessary to detect light, and the signaling pathways required to entrain the oscillator. In fact, in the case of zebrafish, they contain 32 non-visual opsins, divided into four new classes of photopigment (Opn 6-9). We will explore the significance of this opsin diversity, and also examine so other aspects of fish biology that are regulated by this direct light sensitivity, including aspects of daily cell cycle timing. In addition, we will describe how using fish species that have evolved in complete darkness have “shed light” on to the process of cellular light detection. This will include work on the Mexican blind cavefish, *Astyanax mexicanus*, and more recently work on several deep-sea marine species.

SYMPOSIA

Symposium 1: Adult stem cells

S1-01

PHYSIOLOGY OF AN ADULT PERIPHERAL NEUROGENIC NICHE

V. Sobrino, V. Annese, E. Navarro-Guerrero, A. Platero-Luengo, and R. Pardal

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Neural stem cells rise high interest among scientists due to their possible use against neurodegenerative disease. Nervous tissue-specific stem cells reside in specialized niches that allow them to respond to physiological demands. A profound knowledge of the functioning of these niches would allow us to improve the use of stem cells for cell therapy. We have studied the biology of a subpopulation of neural crest-derived stem cells that persist in an adult peripheral chemoreceptor organ: the carotid body. This organ has the capacity to adapt to a hypoxemic situation by increasing in size and neuronal cell number. The carotid body parenchyma contains a subpopulation of neural stem cells able to give rise to neurogenesis in response to hypoxia, activating proliferation and completing differentiation into new neuronal cells. Recent data in our group show that these stem cells are even more versatile, being able to participate also in the angiogenic process that takes place in the organ in parallel to neurogenesis. Carotid body stem cells are able to convert into new vascular cells in response to the hypoxic stimulus. We are studying in detail the biology of this population of adult carotid body neural stem cells. We have identified restricted progenitors within both neuronal and mesenchymal differentiation lineages. Particularly interesting is also the existence of a subpopulation of quiescent neuroblasts, which are able to respond to hypoxia themselves by activating proliferation and maturing to perfectly functional new neuronal cells. We are particularly interested in the intercellular communications by which carotid body germinal niche cells interact to each other, since these contact abilities determine the functioning of the niche and therefore the behavior of stem cells. Altogether, our results on the characterization of the carotid body germinal niche might have high impact on the use of these stem cells for cell therapy.

S1-02

REMOTE SIGNALLING IN NEURAL STEM CELLS MEDIATED BY INFLAMMATION

I. Fariñas, G. Belenguier, J.M. Morante-Redolat, A. Jordán, P. Duarte

Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Estructura de Recerca Interdisciplinaria en Biotecnología i Biomedicina (ERI BIOTECMED), and Departamento de Biología Celular, Biología Funcional y Antropología Física, Universidad de Valencia, Burjassot, Spain

Adult stem cells are found at specific locations and their behavior and lifelong maintenance is regulated by both cell intrinsic factors and signals from the microenvironment or niche in which they reside. However, stem cell niches are still poorly characterized due to the complexity of the interactions between stem cells and their neighbors and to the dynamic changes required for the continuous production of new cells. In the adult brain subependymal zone (SEZ), radial glia/astrocyte-like neural stem cells (NSC) continually produce new neurons and oligodendrocytes, via a

population of rapidly-dividing transit-amplifying progenitor cells. In the adult SEZ, different elements, including innervation, irrigation and the cerebrospinal fluid of the brain lateral ventricles, appear to play important roles in the regulation of NSC behavior, but the signalling pathways involved are still under investigation. Increasing evidence indicates that immune cells and immunological mediators could also modulate NSC behavior. Effects on neurogenesis of pro-inflammatory cytokines that are produced under non-physiological conditions, such as irradiation, inflammation, status epilepticus or stroke, have been described. However, their effects appeared sometimes contradictory, suggesting potentially distinct effects depending on the cell or receptor type involved. Tumor necrosis factor alpha (TNF α), a pro-inflammatory cytokine, is a multifunctional protein with a broad range of activities in different systems. We have evaluated potential roles of TNF α and its receptors in SEZ remodeling/regeneration analyzing direct effects of this cytokine on the proliferation/self-renewal of NSCs in culture and assessing its relevance in different *in vivo* scenarios where SEZ homeostasis is compromised. We have also analyzed the role of the two TNF α receptors using specific TNFR1 and TNFR2 agonists and TNFR knock-out mice. We found that TNF α modulates proliferation, self-renewal and the balance of symmetrical/asymmetrical divisions of NSCs and that each receptor mediates a distinct biological response.

Funding: MINECO (SAF, CIBERNED, and TerCel Programs), Generalitat Valenciana (Prometeo Program), and Botín Foundation-Santander Bank.

Adult neurogenesis, microglia, quiescence, TNFR

S1-03

HORMONAL REGULATION OF BREAST STEM CELLS

M. dM. Vivanco, I. Aurrekoetxea-Rodríguez, M. Rábano, J. San Millán, S. Y. Lee

CIC bioGUNE, Bilbao, Spain

Breast cancer is a very heterogeneous disease, which in part arises from the existence of cells with stem-like properties (cancer stem/progenitor cells, CSCs). The identification of stem/progenitor cells has provided a new understanding of development of resistance to conventional anti-cancer therapies, which continues to be a clinical problem in cancer management. Estrogens are essential regulators of normal mammary gland physiology and are also implicated in breast cancer. Tamoxifen, an antagonist of the estrogen receptor, is used as endocrine therapy, although resistance develops, limiting its success. Normal and cancer stem cells share functional properties, which may be controlled by the same factors. Studying normal breast physiology is essential to understand tumorigenesis. We have shown that stem cells are regulated by hormones and are also involved in the development of resistance to tamoxifen treatment. We have analyzed the effects of hormones in the normal breast and in breast cancer and, in particular, the alterations caused in the stem cell populations. The embryonic stem cell factor Sox2 is expressed in normal breast stem cells and at higher levels in breast tumors and its expression is greatest in tumors that have developed resistance to tamoxifen. We have identified a new regulatory axis that highlights the relevance of Sox family transcription factors as potential therapeutic targets in breast cancer. Understanding the mechanisms of action of Sox2 will provide opportunities for the identification of new therapeutic approaches against hormone resistant breast cancer.

Breast, cancer, estrogen, tamoxifen, resistance

Symposium 2: Organoids

S2-01

HUMAN NORMAL AND TUMOR COLORECTAL ORGANOID: GENE EXPRESSION STUDIES.

A. Barbáchano^(1,2), A. Fernández-Barral^(1,2), A. Costales-Carrera^(1,2), and A Muñoz^(1,2)

⁽¹⁾ Instituto de Investigaciones Biomédicas “Alberto Sols”, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid and IdiPAZ, Madrid, Spain; ⁽²⁾ CIBER de Cáncer (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain

In 2007, Hans Clevers' group identified a stem-cell population in the gut able to renew the entire epithelium every few days. In 2009 this group developed a new 3D culture technology to grow, in an indefinite way, this population of intestinal stem cells from mouse small intestine, and later it was successfully applied to human small and large intestine (colon). These cultures give rise to miniaturized complex structures that resemble the organ and were called organoids or mini-guts. Currently, the enormous increase in this field of research has allowed to obtain organoids from multiple organs (liver, pancreas, breast, lung, prostate...) of different species. Organoids reflect the genomic background, cellular heterogeneity and the structure and functionality of their tissue of origin, are genetically stable and can be obtained from a simple biopsy from patient-derived healthy and diseased tissues. Organoid models are now becoming a promising tool for medical research, disease modelling, drug development, personalized treatment and regenerative medicine and constitute an alternative for animal models. In collaboration with surgeons and pathologists, our lab set up the organoid technology and has established a living biobank of normal and tumor organoids derived from biopsies of colorectal cancer (CRC) patients at Hospital Universitario La Paz in Madrid. By global transcriptomic (RNA-sequencing) analysis we are studying the gene expression profiles of normal and tumor colon organoids and also comparing colon versus rectal organoids from the same individual. In addition, we are studying the response of these human organoids to calcitriol, the active vitamin D metabolite that has putative protective effects against CRC. We are performing chromatin immunoprecipitation-sequencing (ChIP-seq) analyses to identify transcriptional target genes of calcitriol and ultramorphological studies by electron microscopy to examine its effects on the phenotype of organoids cells.

S2-02

PATIENT-DERIVED ORGANOID FOR DRUG SCREENING AND DEVELOPMENT

S. Boj

Hubrecht Organoid Technology (HUB), Yalelaan, Utrecht, the Netherlands

HUB (Hubrecht Organoid Technology) is a non-profit company that was founded by the Hubrecht institute (KNAW) and the UMCU with the aim to translate the Organoid Technology, invented in the lab of Hans Clevers, to preclinical and clinical applications.

Key to the development of the Organoid Technology was the discovery of LGR5+ intestinal adult stem cells by the Clevers lab. When provided with the appropriate growth factors, LGR5+ cells were found to form a polarized epithelium in which stem cells, dividing daughter cells and differentiated cells maintain their natural hierarchical and functional role. Importantly, organoids proved to be both genetically and phenotypically stable during prolonged periods of cell culture and are amenable to all

standard experimental manipulations, including middle through-put drug screen. After the discovery of the method for intestinal cells, we developed methods for many other organs such as breast, lung and pancreas. Proprietary protocols for in vitro expansion of these stem cells from patient tissue constituted the basis for the creation of a ‘Living Biobank’. Because patient relevant model systems are arguably the biggest problem in drug development, the Organoids, which maintain the patient and disease characteristics, have the potential to greatly improve drug development and target identification. At the same time, the Organoids can be used as a next generation personalized medicine model to test effective drug treatments for individual patients in the clinic.

S2-03

UROTHELIAL ORGANOID AS A PLATFORM FOR THE FUNCTIONAL GENOMICS ANALYSIS OF BLADDER CANCER GENES

Francisco X. Real ⁽¹⁾ and collaborators ^(1,2)

⁽¹⁾ Centro Nacional de Investigaciones Oncológicas, Madrid, Spain; ⁽²⁾ Instituto de Investigaciones Biomédicas-Universidad Autónoma de Madrid, Madrid, Spain

The urothelium is a specialized stratified epithelium with unique structural and functional features. Understanding the mechanisms involved in urothelial stem cell biology and differentiation has been limited by the lack of methods for unlimited propagation. This information is crucial to understand the mechanisms involved in bladder cancer development. We have established normal urothelial organoid (NMU-o) cultures from the normal urothelium of wild-type mice and from mice in which new bladder cancer genes have been inactivated. Wild-type NMU-o can be maintained uninterruptedly for >1 year. Organoid growth is dependent on EGF and Wnt activators. High CD49f/ITGA6 expression features a subpopulation of organoid-forming urothelial stem cells expressing basal markers. On induction of differentiation, multilayered organoids show reduced layer number, acquire barrier function, and activate the urothelial program, including expression of uroplakins and tight junction components. Combined pharmacological modulation of PPAR and EGFR was most potent driving cell differentiation. Transcriptome analysis of organoids widely validates the system, highlights the transcriptional networks involved, and reveals NOTCH signaling as a novel pathway required for normal urothelial organoid differentiation. We have also analyzed the properties of NMU-o established from STAG2-deficient and RBM10-deficient mice and will present novel results regarding the effects of inactivation of these tumor suppressors on the behaviour of normal cells. These studies show that NMU-o can be used as a platform for the functional genomics of novel bladder cancer genes.

Organoids, urothelium, cancer, Wnt, Notch, functional genomics

Symposium 3: Innovation in physiology teaching

S3-01

TEACHING: A DRONE’S VIEW OF PHYSIOLOGY

C.H. Ming

Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

The big picture or bird’s eye view of whole body physiology is an essential approach to adopt in teaching physiology. Students learn segmentally when we miss directing them regularly to inter-organ integration that are

operative in vivo. We can e.g. help students to appreciate cardio-respiratory functions for adequate tissue oxygenation, cardio-renal mechanisms in blood volume/pressure regulation, respi-renal linkages in blood pH control. The usual way of dividing ‘short-term’ and ‘long-term’ blood pressure control is convenient but commonly does not promote a unified, wholistic grasp of blood pressure homeostasis. In addition, frequently, physiological events cover more than two organ functions. One good illustration is the activity of the renal sympathetic nerve (RSymN). The autonomic RSymN is linked to the cardiovascular baro-reflex loop. The endocrine hormone/enzyme, renin is released by RSymN and the autonomic nerve also modifies the renal handling of sodium to effect blood volume homeostasis. Conceptual aerial mapping or a drone’s view of physiology will stimulate our students’ thinking and engage them to understand the beautiful balance and designed, integrated functions in the human body.

S3-02

LOW-TECH” FLIPPED CLASSROOM IN PHYSIOLOGY LEARNING

M.D. Ganfomina, S. Díez-Hermano, D. Sánchez

Dpto. de Fisiología, Facultad Medicina, Universidad de Valladolid, Valladolid, Spain

The main goal of our project is to evaluate whether “flipped teaching” works for lecture-size groups of Medical Physiology students. Particularly, we focused in a set of 12 Neurophysiology one-hour sessions, and worked with a class of 50-80 students. In order to avoid “digital divide” limitations, we distributed a low-cost Study Guide Text of Neurophysiology (ISBN: 978-84-8448-955-9) that contains the syllabus core concepts and suitable graphic contents for each class session. On average, each chapter was designed for a student to be able to read it reflexively in less than 40 minutes. Thus, this instructional resource allows students to prepare the contents of the following-day class in advance. No evaluation of the outside-of-the-classroom activity was intended. In-class activities were structured to contain sections that flexibly offer students to discuss “difficult concepts”, “fundamental ideas”, engage in collaborative (think-pair-share) strategies, clinical-case video presentations by volunteer students, concept integration debates, etc. To evaluate this project, we used a satisfaction survey from students as well as a comparative analysis of grade performance between those attending “flipped classrooms” (two years) and those attending standard lectures (five years). Our results show that students evaluate positively the resources used and the overall strategy. Besides increasing attendance and participation, the “flipped-classroom students” also showed significantly higher grades. These results encourage us to continue our project to fine-tune the method and explore better ways to improve our Physiology students learning experience.

Teaching Strategy, Problem Solving, Active Learning

S3-03

THE WSLA, A MODEL TO LEARN PHYSIOLOGY IN AN INTEGRATED MANNER

B. Gal, A.M. Sánchez, A.I. R-Learte, R. González-Soltero

Departamento de Ciencias Biomédicas Básicas, Universidad Europea de Madrid, Villaviciosa de Odón, Madrid, Spain

The need to integrate knowledge and skills in higher education has become an essential demand, especially in the biomedical sciences. Establishing integrated programs in medical education is, however, a challenge. Recently, at the European University of Madrid we have opted to tackle a progressive integration, without needing to get to the curricular level, through a new model called WSLA (Work Stations Learning Activities). The WSLA serves to create integrated learning modules that can be applied and adapted to different situations, from master classes to laboratory practices. This new approach was conceived at two different levels: first, we identified potentially integrative units from different fields according to national learning goals established for each preclinical year (national quality agency regulations). Secondly, we implemented a new instrument that combines active methodologies in Work Station Learning Activities (WSLA), using clinical scenarios as a guiding common thread to instruct students from an integrated perspective. We evaluated students' perception through a Likert-type survey of a total of 118 students enrolled in the first year of the Bachelor's Degree in Medicine. Students' perception of WSLA was positive in overall terms. Seventy nine percent of participants stated that WSLA sessions were more useful than non-integrated activities. Eighty three percent confirmed that the WSLA methodology was effective at integrating concepts covered by different subjects. The WSLA approach is a flexible and scalable instrument for moving towards integrated curricula, and it can be successfully adapted to teach Physiology in preclinical years of Health Science degrees. WSLA can be applied to large groups of students in a variety of contexts or environments using clinical cases as connecting threads.

WSLA, Integrated curriculum, Biomedical schools Background

S3-04

THE IMPACT OF MULTIPLE CHOICE QUESTIONS IN PHYSIOLOGY LEARNING. HOW TO WRITE MULTIPLE CHOICE QUESTIONS IN PHYSIOLOGY

Jorge J. García Seoane

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In recent generations, objective evaluation has taken over subjective evaluation as the main examination tool. Standardized, multiple-choice testing has become the main contributor to final course grades and it is therefore impossible to prevent students from preparing for these exams by conducting drills with questions from previous years. The use of the classic multiple-choice test type in which test takers select the correct answer can have the undesirable effect of generating confusion in the students who learn through simulated drill repetition. Students are reading very few true concepts mixed with many false concepts associated with a question. Over time, memory tends to interpret everything we read as true simply because it sounds familiar. During the 2016-17 academic year we conducted an experimental study with the hypothesis that practice test repetition would generate both learning and confusion. The volunteer student participants completed self-assessment exams, in the virtual campus, during the month dedicated to endocrinology study. Of the 30 true / false questions of the sanctioning test, 15 had already been used in the self-assessment exams. The students who participated in self-assessment drills were classified according to the correct or incorrect answers in the self-assessment, and later in the exam. Overall, the students who participated in the experience answered better than the students who did not participate. However, they fail more in the questions presented as false in the final exam, marking some of them as true. This effect is seen most clearly in the questions that were answered incorrectly during the self-assessment, in which the percentage of failure is approximately 50% and is even higher than that of the students who did not participate in the

experience (35% failure), which indicates that the participant students transformed into true a high proportion of the questions that they saw presented as false during the self-evaluation. Although it is still essential that the evaluation process includes different types of testing, for the part that is a standardized, multiple choice test, the best option would be questions with 4 different answers (A, B, C, D), but where students are required to select the false answer in the group, so that most of the options they read are correct. Some could even have all correct answers and an extra null answer to be marked. We will discuss different models of improvement so that the test type evaluation minimizes the confusion effect and reinforces correct learning.

Symposium 4: Immunomodulatory and antioxidants effects of melatonin

S4-01

EFFECT OF MELATONIN ON THE PRESERVATION AND SYNTHESIS OF PROTEINS UNDER STRESS CONDITIONS.

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Although physiological oxidative stress level promotes protective functions to improve adaptive stress response, persistent high oxidative stress cause irreparable cell damage and can lead to pathological states. Protein synthesis is an important complex process indispensable for the maintenance of cellular homeostasis, which in response to stress conditions is inhibited. We have extensive experience in studying the effect of oxidative stress-induced lipid peroxidation on protein synthesis through mechanisms that involve regulation of eElongation Factor 2 (eEF2). In fact, we have found that in response to oxidative stress, eEF2 could be altered and thereby promote the inhibition of global protein synthesis and stimulate selective translation of specific proteins that, under oxidative stress conditions, promote cell survival. Due to eEF2 is particularly sensitive to increased oxidative stress, we studied the antioxidant effect of melatonin in preventing eEF-2 oxidative changes and protein synthesis inhibition. We found that melatonin was able to prevent partial- or totally the alterations of eEF2 in liver, hypothalamus, hypophysis and pineal gland in response to oxidative stress using young rats exposed to oxidative stress-induced lipid peroxidation. These results suggest that melatonin may be used to increase preservation of eEF2 and reduce protein synthesis inhibition under oxidative stress conditions.

Melatonin, protein synthesis, oxidative stress

S4-02

IMMUNOMODULATORY EFFECTS OF MELATONIN

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Multiple sclerosis (MS) is a multifactorial disease that currently has no cure. The complex pathological mechanisms that underlie this disease, which include inter-relating inflammatory and

neurodegenerative processes, has led to search for a treatment with immunomodulatory, antioxidant and neuroprotective effects. Despite the large number of reports implicating melatonin as an immunomodulatory compound, it remains unclear how melatonin regulates immunity. While some authors argue that melatonin is an immunostimulant, many studies have also described anti-inflammatory properties. We support the idea of melatonin as an immune buffer, acting as a stimulant under basal or immunosuppressive conditions or as an anti-inflammatory compound in the presence of exacerbated immune responses, such as acute inflammation. In the present study, we describe how melatonin controls experimental autoimmune encephalomyelitis (EAE), the animal model for MS, a neuro-inflammatory disease triggered by over-reacting myelin-specific Th1 and Th17 cells. We seek to elucidate the basis of the melatonin protective role in EAE by characterizing the T effector/regulatory responses, particularly those of the memory cell subsets. Melatonin was tested for its effect on Th1, Th17 and T regulatory (Treg) cells in the lymph nodes and CNS of immuno-dominant peptide of myelin oligodendrocyte glycoprotein (pMOG)-immunized and EAE mice, respectively. Melatonin protected from EAE by decreasing peripheral and central Th1/Th17 responses and enhancing both the Treg response and IL-10 synthesis in the CNS. Moreover, we show the protective effect of the combined treatment with melatonin and methylprednisolone on the development of EAE and the effectiveness of innovative nanocarriers for improving melatonin bioavailability. Additionally, we display the melatonin effects on the inflammatory response in circulating lymphocytes of MS patients. In conclusion, the melatonin immunomodulatory role in EAE and immune cells from MS patients suggests that melatonin may represent an effective treatment option for MS.

Melatonin, multiple sclerosis, immunomodulation, Th1, Th17, T regulatory cells

S4-03

EFFICACY OF A NEW PHARMACEUTICAL FORMULATION OF MELATONIN TO PREVENT CELL DAMAGE

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Melatonin, a hormone produced by the pineal gland, has been detected in multiple extrapineal organ tissues at much higher concentrations than in the pineal gland. It is a potent free radical scavenger with anti-oxidant properties, which increases the expression and activity of endogenous antioxidant. This special class of antioxidant generates a series of metabolites that are also free radical scavengers when scavenging free radicals. Capable of crossing cell membranes and of easily reaching all cell compartments, it is taken up by mitochondria and can maintain mitochondrial homeostasis in different experimental models. Melatonin has also important anti-inflammatory effects. Recently, we have developed a pharmaceutical preparation of melatonin plus other molecules for the treatment and prevention of skin aging. The success of this preparation is that the composition facilitates their transdermal adsorption, reaching both molecules all skin's layers. Moreover, the combination of both molecules increases their take up by the mitochondria in all skin cells. The advantage of our product is that not only reverse the mitochondrial damage produced during cellular aging but also in many pathologies coursing with mitochondrial impairment.

Melatonin, oxidative stress, skin aging.

Symposium 5: Exercise for the prevention and treatment of cardiovascular disease

S5-01

CARDIORESPIRATORY FITNESS AND MUSCULAR STRENGTH IN YOUTH AS PREDICTORS OF FUTURE CHRONIC DISEASE

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From the landmark studies of Prof. Paffenbarguer and Blair in the 70's and 80's to date, an enormous amount of evidence has accumulated supporting a strong link between the cardiorespiratory fitness (CRF), and more recently also muscular strength (MST), in adulthood and overall and specific morbidity and mortality. Several longitudinal studies also showed that physical fitness (both CRF and MST) in childhood and adolescence are associated with lower risk factors for cardiovascular diseases and other health outcomes. However, whether physical fitness assessment at these young ages directly predict future mortality and morbidity was unknown, since very large cohorts and with a very long follow-up are needed to test these hypotheses and it has been a lack of such studies. During my presentation in this scientific event, I will cover this topic, presenting the state of the art in relation with fitness in youth and future health. I will discuss some of the most powerful and recent studies, as well as share with the audience some preliminary unpublished findings.

S5-02

EXERCISE AND THE MEDITERRANEAN DIET FOR THE PREVENTION AND TREATMENT OF CARDIOMETABOLIC ABNORMALITIES INDUCED BY MENOPAUSE. THE FLAMENCO STUDY

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Background: Menopause increases cardiometabolic risk due to the decline in oestrogens. In this sense, increasing fitness through exercise and the Mediterranean Diet (MD) may promote positive effects on several cardiometabolic markers in this physiological stage. AIMS: i) To examine the association of sedentary time (ST) and physical fitness with “Ideal Cardiovascular Health” (ICH) in perimenopausal women from “the Fitness League Against MENopause CoSt (FLAMENCO)” project; ii) To explore the influence of the MD adherence on a set of cardiometabolic markers; iii) To investigate the influence of a concurrent exercise training on several markers of cardiometabolic risk; iv) To explore the influence of this exercise program on dietary habits.

Methodology: The cross-sectional analyses included 198 perimenopausal women recruited for the Flamenco project. From them, 150 (52.2 ± 4.4 years) were randomized to a four-month concurrent (aerobic + resistance) exercise intervention, or healthy life-style counselling group. Body composition was estimated through DXA. Questionnaires were used to assess

the degree of adherence to the MD. The following cardiometabolic risk factors were assessed: BMI, fat mass, waist circumference, blood pressure, resting heart rate and plasma total cholesterol, high and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively), triglycerides, C-reactive protein and fasting glucose. ST and physical activity were objectively assessed. Physical fitness was assessed with the Senior Fitness Test battery, handgrip strength and sit-and-reach tests.

Results: After adjusting for covariates, perimenopausal women with ICH showed lower ST and greater cardiorespiratory fitness and upper-body flexibility compared to women with a non-ICH status (all, $p < 0.05$). Women with a high adherence to the MD showed lower resting heart rate ($p = 0.005$), total cholesterol ($p = 0.025$), LDL-C ($p = 0.019$), triglycerides ($p = 0.046$) and C-reactive protein ($p = 0.009$). Regarding the Flamenco project intervention, the exercise compared to the counselling group decreased BMI ($p = 0.006$), gynoid fat mass ($p = 0.007$), android fat mass and pelvis bone mineral content (both, $p < 0.05$). The exercise group also showed lower diastolic blood pressure and LDL-C (both, $p < 0.05$). However, women in the exercise group significantly increased their beer intake.

Conclusion: Including ST, cardiorespiratory fitness and upper-body flexibility as complementary ICH metrics might improve early identification of climacteric women at high cardiovascular risk. Moreover, a high but not medium adherence to the MD may be cardioprotective. The Flamenco project training protocol preserved bone and decreased central fat and BMI. This training program might slightly improve vascular and lipid profile beyond the life-style counselling. Future studies should combine this concurrent exercise protocol with dietary interventions based on the MD in search of better results.

Physical fitness; climacteric; inflammation; lipid profile; glycaemic profile

S5-03

ROLE OF HUMAN BROWN FAT IN CARDIOVASCULAR DISEASE: STRATEGIES TO TURN UP THE HEAT

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Human BAT was re-discovered in 2009 by several independent groups, who showed that it is present and active in adults, as judged from the profound uptake of the glucose analogue radiotracer 18F-fluorodeoxyglucose in positron-emission tomography and computed tomography scan analysis after cold exposure. A potential clinical implication of activating BAT relates to its high metabolic activity and its potential role in stimulating energy expenditure (i.e. basal energy expenditure, diet-induced thermogenesis, and cold-induced thermogenesis), which makes it an attractive target to reduce adiposity. Moreover, due to its ability to oxidise glucose and lipids, BAT activation also exerts beneficial metabolic and cardiovascular effects through reducing glucose and lipid levels, respectively. This review describes the potential role of human BAT in the prevention and treatment of cardiovascular disease focusing on its impact on energy expenditure and management of body fat

accumulation as well as on glucose and lipid metabolism. This article also summarises the strategies that are currently being studied to activate human BAT.

18F-fluorodeoxyglucose, positron-emission tomography, cold-induced thermogenesis, exercise, diet-induced thermogenesis

Symposium 6: Current regulation and new trends in animal research

S6-01

NON-INVASIVE IMAGE TECHNIQUES IN ANIMAL EXPERIMENTATION

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Following the objectives of both Directive 2010/63 / EU and Royal Decree 53/2013, we seek the use of non-invasive imaging techniques to optimize the use of experimental animals by reducing the number of animals and refining the techniques used in the experimental procedures. Through non-invasive imaging techniques we can "reduce" the number of animals by allowing the same animal to be used as a control and as an individual study at different times of the procedure. At the same time, this allows us to know at every moment the progress of the implanted pathology, thus providing a deeper supervision of each individual. Optical imaging in animals is based on using photons of light to obtain images. For in vivo studies, we can use bioluminescence and fluorescence. Thanks to the development of very sensitive cameras such as CCD (charged coupled devices), it has been possible to transfer for in vivo use techniques in principle only viable in microscope images. Fluorescent probes for in vivo use of near infrared (NIR) emissions have a greater penetration into biological tissue. Another imaging technique used in experimentation is the PET / CT camera. Computed tomography (CT) is a medical imaging technique that uses X-rays to obtain slices or sections of anatomical objects for diagnostic purposes. Positron emission tomography (PET) is a nuclear medicine imaging technique that takes advantage of the emission of positrons by certain radioactive elements. The fusion of images from the PET / CT camera allows us to superimpose anatomical images and images of cellular function (or metabolism) obtaining a greater diagnostic accuracy and greater ease of interpretation. Another option of "refinement" in experimental surgery that should be taken into account is the use of an eco-guided vascular access technique, reducing the incidence of complications. The use of diagnostic equipment by scotopic imaging is a direct vision system in explorations with contrast, which makes it possible to obtain experimental models, such as an acute myocardial infarction in porcine model, utilising a less traumatic methodology

Reduction, refinement, non-invasive image

S6-02

GENETIC MODIFICATION: REFINING ANIMAL MODELS FOR RESEARCH

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Animal models have played a pivotal role in biomedical research. However, the information obtained has been restricted by specific

physiological characteristics of the model that sometimes barely resemble those of the specie studied, mostly human diseases. In other situations, the scientific hypothesis simply could not be tested due to lack of the proper model. Genetic engineering has provided the tools to modify genetic information adapting the model to specific genotypic characteristics more similar to the target specie. Humanized mice are the perfect example of this approach and represent only a small amount of thousands of models already created for different purposes. When proposing a new research project involving GA animals, it is important to check if the required model is available or if it should be created de novo. This presentation pursues two goals: a) to summarize technologies available for the creation of genetically altered animal models: transgenesis, gene targeting and genome editing. The different application of each technology will be also discussed, and b) to present resources available to find the desired model and the international consortia distributing them. The use of these resources allows a reduction of animals needed. To determine if a given animal model is available, is a mandatory first step in any project proposal based on the use of genetically altered animals in order to avoid unnecessary duplications, and these international databases and consortia are a unique source of relevant scientific information.

Genetically altered animal models, transgenesis

S6-03

HOW THE 3RS (REPLACEMENT, REDUCTION AND REFINEMENT) CONTRIBUTE TO IMPROVE THE QUALITY OF ANIMAL STUDIES

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The 3Rs are widely accepted as the ethical principles for the conduct of research involving animal models. In Europe, these principles were implemented by European Directive EU63/2010. **Replacement:** While there are a growing number of alternatives to animal models, some of them including several tissues together, we are still far from being able to completely substitute live animals by in-vitro models. However, the search for alternatives over the years has allowed the development of simpler vitro systems that help to understand life systems, and save time, resources and animal lives. **Reduction:** This principle should be understood not only as using as few animals as possible but also in order to define the best possible experimental design that will give robust results. We should also take into consideration, the correct estimation of the number of animals needed in a study, the use of pilot studies when necessary and, even more important, to correctly randomize in all phases of the study. **Refinement:** Technology is helping a lot in the continuous refinement in animal experiments. The contribution of imaging techniques reduces both the pain and distress to animals and improves the reliability and reproducibility of results. For example, imaging techniques allow the study of the evolution of a treatment in the same animal (also contributing to Reduction) and reduce variability. However, good training of the person conducting a surgery and aseptic surgery, although done in the lab, etc. also contribute very significantly to improve the welfare of the animals as well as the results we obtain from them. Finally, it is important to pay attention to the quality of the reporting of scientific results. The use of tools such as ARRIVE guidelines when designing an experiment and when preparing a manuscript will help to avoid the repetition of experiments as well as facilitate the translation of animal studies to human health. Fortunately, one of the tools we have to transmit these ethical principles to young scientists is by means of the training courses they have to complete before they become accredited to work with animal models.

S6-04

SPANISH REGULATIONS FOR THE PROTECTION OF ANIMALS USED WITH SCIENTIFIC PURPOSES

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National basic regulation on the use of animals for scientific purposes in Spain responds to the obligation to transpose into national legislation the Directive 2010/63 / EU of the European Parliament and of the Council of September 22, relative to the protection of animals used for scientific purposes. Rules are, on the one hand, Law 32/2007, of November 7, for the care of animals, in their exploitation, transport, experimentation and killing, and Royal Decree 53/2013, which establishes the basic rules applicable for the protection of animals used in experimentation and other scientific purposes, including teaching. Some aspects of those regulations have a particular development, such as issues related to staff education and training, which is materialized through Order ECC / 566/2015, of March 20, which establishes the training requirements to be met by personnel handling animals used, bred or supplied for experimental and other scientific purposes, including teaching. On the other hand, and given the distribution of competences between the general administration of the state, and the regions, regions have the possibility to develop their own regulations, which adapt the requirements to regional particularities that may exist. Due to this normative *acquis*, the use of animals in this context is strictly regulated. Use is limited to situations that require a detailed authorization process that affects both the environment in which they will be bred, supplied or used (establishments), the people directly and indirectly involved in that use, the way in which they are used (research projects, maintenance conditions, killing systems ...), and, of course, the animals themselves. The regulations also require an exhaustive recording of regulated activities and assure access to information of interested citizens by the publication, on the one hand, of the so-called non-technical summaries of authorized projects, and on the other, of statistical information on the uses of animals in this context in each Member State. In the short and medium term, two modifications are foreseen in Royal Decree 53/2013, of 1 February, one to clarify some issues, and the other because of the Proposal for a regulation regarding the adaptation of notification notifications in the field of the environmental policy.

Symposium 7: Cardiovascular risk from basic to clinical research

S7-01

CRITICAL ROLE OF THYROID STIMULATING HORMONE (TSH) IN CARDIAC ELECTRICAL REMODELING AND ARRHYTHMIA VULNERABILITY IN HYPOTHYROIDISM

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Hypothyroidism, the most common endocrine disease, induces a cardiac electrical remodeling, creates a proarrhythmic substrate and facilitates the occurrence of ventricular arrhythmias including torsades de pointes. Recent studies highlight the importance of Thyroid-Stimulant Hormone (TSH) levels in the mortality of both hypothyroid patients and euthyroid individuals. We used animal models of primary (high TSH) and central (low TSH) hypothyroidism, human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CM), adult human atrial myocytes, rodent ventricular myocytes and computational modeling to gain insight into the mechanisms underlying myocardial electrical remodeling by TSH. The results suggest that the cardiac electrical remodeling and arrhythmia susceptibility in hypothyroidism are due to: i) an increase of I_{Ca-L} secondary to the reduction of thyroid hormone levels; ii) a TSH-dependent reduction of the expression of Kv4.3 and Kv7.1 (pore-forming proteins of the channels that carry I_{to} and I_{Ks}, respectively) and of the Na⁺/Ca²⁺ exchanger activity; and iii) appearance of early afterdepolarizations and arrhythmias secondary to prolongation of repolarization, especially under β -adrenergic stimulation. In summary, this work demonstrates the direct involvement of TSH in the cardiac electrical remodeling and arrhythmia generation seen in hypothyroidism.

Ionic currents, cardiac electrophysiology, repolarization

S7-02

ELECTRICAL REMODELING IN ESSENTIAL HYPERTENSION: VASCULAR ION CHANNELS REDRESS THE BALANCE

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The contractile state of the vascular smooth muscle cells (VSMCs) of the vessels wall is modulated by a complex interplay of vasodilator and vasoconstrictor stimuli to determine vascular tone. Changes in this equilibrium during hypertension lead to the hypertrophic inward remodeling of resistance arteries. Together with this mechanical remodeling, there is an adaptive “electrical remodeling” of VSMCs, creating a disease-specific profile of vascular ion channels. By controlling resting membrane potential and cytoplasmic [Ca²⁺] in VSMCs, ion channels are relevant in all aspects of contractile vascular function. A sustained Ca²⁺ influx through voltage-dependent Ca²⁺ channels (VDCC) is required for vasoconstriction. This determines that depolarization of VSMCs is a common feature of hypertension models. However, due to the large diversity of ion channels present in VSMCs the underlying molecular mechanisms are poorly defined. Using a hypertensive mice strain (BPH) and its corresponding normotensive control (BPN), we have created a global portrait of ion channels genes from several vascular beds, and we identified several changes in mRNA expression of ion channels in hypertensive vessels. We have found changes in the functional contribution of VDCC, that in coordination with changes in several types of K channels and transient receptor potential (TRP) channels contribute to the hypertensive phenotype. Also, our results stress out two important lessons: First, that the changes in the expression profile must be interpreted in an integrated, global context to properly understand the phenotypic changes, and second, that in order to determine the role of these changes in the development of hypertension we need to define their contribution to cell excitability and vascular tone.

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S7-03

A NOVEL ROLE OF FIBROBLAST GROWTH FACTOR (FGF)-23 IN VENTRICULAR ARRHYTHMOGENESIS

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Cardiovascular (CV) disease is the number one cause of morbidity and mortality in dialysis patients. In these patients, two thirds of cardiac deaths are attributed to cardiac arrhythmia, and sudden cardiac death (SCD) accounts for approximately 25% of the total deaths. Despite the prevention of these fatal arrhythmic events is crucial to improve the survival of dialysis patients, unfortunately, the underlying mechanisms associated with them are not elucidated yet. Fibroblast growth factor (FGF)-23 is a hormone synthesized in bones in response of an increase in circulating phosphate levels. It is known that patients with chronic kidney disease (CKD) show high serum levels of FGF-23 and this increment is gradual as CKD progresses. Despite of FGF-23 has been classically associated to renal dysfunction, during the last years is also being considered as a non-conventional risk factor of CV disease. The purpose of this study was to analyze whether FGF-23 impairs calcium (Ca²⁺) handling and consequently the ventricular cardiomyocyte rhythm. Enzymatically isolated adult rat ventricular myocytes were perfused firstly with a vehicle solution and subsequently with a FGF-23 solution (100 ng/mL). L-type Ca²⁺ current (I_{CaL}) was recorded by the whole-cell patch-clamp technique. Ca²⁺ handling and contractile function were analyzed using confocal microscopy. To determine FGF-23-dependent pathways, cardiomyocytes were preincubated with the FGF-receptors inhibitor PD173074 (10 μ mol/mL) or soluble klotho (s-klotho) (100 ng/mL). FGF-23 induced a significant decline of I_{CaL} (p<0.001), an important decrease in the intracellular Ca²⁺ transients amplitude (p<0.01) and in the sarcoplasmic reticulum Ca²⁺ load (p<0.01). All these alterations were functionally translated to a significant deterioration of cellular contraction values (p<0.01). Additionally, a considerable increase in diastolic Ca²⁺ sparks and waves (p<0.01) were observed during FGF-23 exposure. Interestingly, during FGF-23 perfusion cardiomyocytes showed a pro-arrhythmogenic phenotype when they were electrically stimulated. These effects induced by FGF-23 were blocked when cardiomyocytes were pretreated with PD173074 or s-klotho. Our study uncovers FGF-23 as a new target in the intracellular Ca²⁺ handling, able to impair contractile function and induce a pro-arrhythmogenic phenotype in adult cardiomyocytes. Alterations evoked by FGF-23 in cardiomyocytes could explain the CV events observed in patients with CKD, especially those in dialysis.

FGF-23, klotho, ventricular cardiomyocytes, Ca²⁺ handling, arrhythmia

Symposium 8: Obesity and adipose tissue: new perspectives

S8-01

OBESITY AND ADIPOSE TISSUE REGULATION BY THE CDK2-P27 AXIS

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Obesity is a global health problem which affects millions of people worldwide and has nearly tripled its prevalence. It is characterized by an excessive accumulation of fat in adipose tissue, this increases the risk of suffering a series of metabolic diseases such as type 2 diabetes, dyslipidaemia and atherosclerosis. Recently, a family of protein kinases called cyclin dependent kinases (CDKs) has emerged as a possible mediator in obesity. CDKs and other members of the cell cycle machinery not only take part in cell cycle progression, but also in metabolic processes like insulin secretion in pancreas, gluconeogenesis in liver or insulin resistance in adipose tissue. The aim was to understand if the CDK2-p27 axis plays a function in the metabolism of mature adipocytes and consequently in obesity. An analysis of mRNA expression in human adipocytes isolated from subcutaneous adipose tissue shows that CDK2 expression is augmented with obesity. Moreover, *in vivo* studies in mice models indicated that CDK2 protein level is increased in diet-induced obese mice, thus, suggesting a likely function regarding metabolism. In fact, this role has been confirmed in genetic modified mice models, affecting the body weight, the adipose mass, and the adipose tissue function (white and brown). Furthermore, *in vitro* studies with cultured mature adipocytes and treated with a CDK2 inhibitor, showed that CDK2 was involved in regulation of insulin sensitivity, induction of glucose transport and glycerol release. Together these results indicate that the CDK2-p27 axis as a major regulator of the adipose metabolism which can be applied to optimize the design of clinical approaches and the discovery of new pharmacological treatments against obesity and its associated comorbidities.

S8-02

ADIPOSE TISSUE AND BIOLOGICAL CLOCK

Garaulet M^(1, 2)

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Chronodisruption (CD) is defined as the desynchronization of the 24-h rhythms, reductions in the amplitude of these rhythms, or a combination. It is also known that desynchronization between external environmental cues and internal physiological processes can also result in a reduction in rhythm amplitudes. Furthermore, CD can also be produced by alteration of the core machinery of the molecular circadian clock. Brain- and muscle ANRT-like protein-1 (BMAL1) is one of the key core clock elements in the biological clock. In addition to its role in this circadian molecular clock, it has a specific role in the organism's physiology. Animal models have shown that mice with *Bmal1* disruption are prone to developing metabolic disorders. A hallmark of obesity is the excessive expansion of body fat (i.e., adipose tissue) which can be due to an increase of fat cell size, fat cell number or both. Compared to normal-weight, overweight/obese subjects display reduced amplitude rhythms of several adipokines in plasma values and AT gene expression. It has been suggested that alterations in the molecular clock mechanism within the adipocyte may induce metabolic changes that can disrupt metabolism, induce adipose accumulation and lead to obesity. Nevertheless, little is known regarding the peripheral clock molecular mechanisms that influence these events. In humans, the study of adipose rhythms is challenging. The classical circadian rhythm experiments are performed with AT biopsies series or cultured explants. However, due to the large amount of tissue

required, such studies only permit a limited number of measurements to be performed (4-6/24h). Another critical aspect of these circadian experiments is that they do not allow following the same cells over time. Recently, we have implemented a new technique to study human AT circadian rhythms by bioluminescence using a lentiviral approach to introduce a *BMAL1:luciferase* reporter into human adipocytes. This technique has allowed us to continuously measure expression in the same AT cells for at least three days with high temporal resolution.

S8-03

TARGETING BROWN ADIPOSE TISSUE IN OBESITY AND DIABETES

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Obesity is growing at an alarming rate and in parallel to the increase of its associated metabolic diseases such as type 2 diabetes, insulin resistance, cardiovascular disease and some forms of cancer. The discovery of active brown adipose tissue (BAT) in adult humans and that it is reduced during obesity and diabetes has put a spotlight on this tissue as a therapeutic target in obesity-induced metabolic disorders. As opposed to the energy-storing white adipose tissue (WAT), BAT utilizes fuels such as fatty acids to produce heat and control body temperature in a process called thermogenesis. The mechanisms involved in the development of obesity-induced metabolic diseases have been associated to WAT dysfunction such as fibrosis, apoptosis, inflammation, ER and oxidative stress. However, little is known of whether these processes are also present in BAT during obesity. Our aim was to characterize the BAT of obese and hyperglycemic mice treated with a high-fat diet (HFD) for 20 weeks. The hypertrophic BAT from obese mice showed elevated levels of inflammation, ER stress, ROS generation and antioxidant enzyme activity compared to lean controls. The response was attenuated compared with obesity-induced WAT derangements, which suggests that BAT is more resistant to the obesity-induced insult. In fact, mitochondrial respiration measured with the Seahorse analyzer in BAT from obese mice was enhanced, with a 2-fold increase in basal oxygen consumption, through the upregulation of complex III of the electron transport chain and UCP1. In conclusion, our results show that obesity is accompanied by an increase in BAT mitochondrial activity, inflammation and oxidative damage.

Obesity, brown adipose tissue, thermogenesis, inflammation, oxidative damage

S8-04

BIOACTIVE LIPID MEDIATORS AS REGULATORS OF ADIPOSE TISSUE FUNCTIONS IN OBESITY

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Obesity is considered a chronic low-grade inflammatory disease. This inflammatory state associated with obesity appears to be linked with several obesity-related disorders such as insulin resistance, cardiovascular diseases, immune dysfunction, and certain types of cancer. Adipose tissues play crucial roles in the development of obesity, with white adipose tissue (WAT) functioning as an energy storage organ and brown adipose tissue (BAT) as an energy-dissipating organ. Low-grade chronic inflammation in WAT plays a key role in the pathophysiology of obesity. Inflamed WAT exhibit an altered secretory pattern characterized by increased secretion of pro-inflammatory adipokines, cytokines and chemokines, and reduced production of anti-inflammatory adipokines. Progressive infiltration and activation of M1 proinflammatory macrophages and T/B cells also occurs within hypertrophied WAT. The pro-inflammatory obese environment also alters BAT and beige metabolism and thermogenic function. Lipids are bioactive molecules with capacity to trigger profound physiological responses. Indeed, bioactive lipids such as eicosanoids, ceramides and fatty acids have been shown to control important cellular processes in adipose tissue, including adipogenesis, metabolism and inflammation. Resolution of inflammation is an active process, which involves production of several series of specialized proresolving lipid mediators (SPMs), mainly derived from n-3 polyunsaturated fatty acids, such as resolvins, protectins and maresins. Obesity is accompanied by an impaired adipose tissue local production of some of these SPMs. In contrast, preclinical studies have unraveled the therapeutic potential of some of these SPMs for reversing adipose tissue inflammation and insulin-resistance in obese rodents.

Symposium 9: Setting the biological clock and the importance of non-visual light detection in fish

S9-01

HORMONAL INPUTS FOR FISH CIRCADIAN SYSTEM: GOLDFISH AS A MODEL

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The circadian system is responsible for the temporal organisation of behavioural, metabolic and physiological, functions which, in part, involves daily cycles of hormonal activity. This system consists of a net of central and peripherally located oscillators, which operate by transcriptional-translational loops of the called clock genes. The light-dark cycle is the most important external zeitgeber for the vertebrate circadian system, but food availability and temperature cycles are also important. These environmental factors are considered the 'inputs' of the clocks, while the rhythms that are generated are called the 'outputs' or 'overt rhythms'. In addition to these external synchronizers, more recently it has been proposed that hormones, as cortisol, can also act as inputs for other endogenous oscillators in mammals. However such possibility has been almost unexplored in fish, which have a less hierarchical circadian organization than

mammals being the putative role of hormones as temporal messengers more relevant to keep temporal homeostasis. In our group we have analysed the interplay between the circadian and endocrine systems in goldfish focussing in cortisol and ghrelin. We have studied their role as overt rhythms (i.e. outputs of the circadian system) and, for the first time, as key internal temporal messengers that act as inputs for other endogenous oscillators. *In vivo*, cortisol induces the clock genes *per1a* and *per1b* (probably by a Glucocorticoid Response Element in *per1* promoters), while repress *clock1a* and *bmal1* in goldfish. These results have been also shown in hepatic cultures *in vitro*. On the other hand, ghrelin induces the mRNA expression of several clock genes in cultured goldfish liver via protein kinase C and protein kinase A pathways. Based on these acute changes in clock gene expression, we propose a model for non-photic (endocrine) entrainment. Our results highlight the importance of the bidirectional crosstalking between the endocrine and circadian systems in fishes. The flexibility of the fish circadian system combined with the absence of a master clock makes these vertebrates a very attractive model for studying communication among oscillators to drive functionally coordinated outputs.

This research was supported by the Ministerio de Economía y Competitividad [project MINECO; AGL2016-74857-C3-2-R].

S9-02

BIOLOGICAL RHYTHMS OF REPRODUCTION IN VERTEBRATES: APPLICATION TO ASSISTED REPRODUCTION TECHNOLOGIES

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Many variables in the natural environment such as light and temperature display cyclic and predictable variations. To adapt to these cyclic changes, animals present biological rhythms in many of their physiological processes, timing their functions to occur when the possibility of success is the greatest. Among these, many processes of reproduction in vertebrates have been described to display daily and seasonal rhythms. However, application of this knowledge to reproduction techniques such as *in vitro* fertilization or embryo and larval rearing remains little explored to date. Our research group has recently described the existence of a circadian rhythm of *in vitro* fertilization in the zebrafish (*Danio rerio*), which presents the greatest success around the end of the night and beginning of the light phase. This coincides with the time of day in which zebrafish breed under natural conditions. In a second experiment, we identified the oocyte as the factor that determines the existence of this daily rhythm of fertilization and deepened in the molecular factors involved like the proteins of the chorion and the calcium wave. In addition, we have evaluated the importance of cyclic environmental factors (light- and thermo-cycles) on embryo and larval development through a comparative study in fish (zebrafish) and mammals (mouse). In both experimental models, the best developmental rates were found when the environmental conditions were cyclic and thus more similar to the natural conditions. The results presented here highlight the importance of considering biological rhythms and a cyclic environment when dealing with vertebrate reproduction. Furthermore, they could help to improve some techniques used in assisted reproduction such as *in vitro* fertilization.

Reproduction, fertilization, biological clocks, light, temperature, development

S9-03

EMBRYONIC CELL LINES FROM MARINE FISH AS TOOLS TO STUDY CELLULAR CLOCKS

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Circadian clock function relies on interlocked transcriptional-translational feedback loops that drives most physiological, cellular, and behavioural processes. Vertebrate circadian systems include a network of endogenous clocks across organs and tissues synchronized one to another and to environmental cues, representing the light (L)-dark (D) cycle the most prominent entraining factor. It seems that circadian rhythms are cell-autonomous mechanisms, as evidenced by the presence of cellular circadian oscillations in clock and clock-controlled genes reported in zebrafish (*Danio rerio*). However, cellular clocks have been poorly studied so far, especially in fish. In this sense, new cell lines from diverse species are promising experimental models for enlarging our knowledge about the organization of cellular circadian clocks. In a previous work, we developed two monoclonal embryonic cell lines (SAEC-A3 and SAEC-H7) derived from the blastula stage embryos of seabream, *Sparus aurata*, a highly commercial marine teleost fish. In the present study, we report the initial characterization of some molecular clock genes (clock, bmal1, per2 and cry1) rhythms and its regulation by light in these seabream cell lines, by using real time quantitative PCR. Moreover, the effect of light-dark cycles on cell proliferation was studied by analyzing the expression of the proliferating cell nuclear antigen (pcna). Several experiments with different photoperiod regimes were conducted: 1, cycle inversion LD-DL; 2, LD followed by constant light (LL), and 3, LD followed by constant dark (DD). Total RNA was harvested every 4–6 h during 24h-cycles and 500 ng were retrotranscribed to cDNA for amplification. Our results showed that both SAEC-A3 and SAEC-H7 rhythmically expressed key clock genes, with acrophases placed during the day-night transition for clock and bmal1, and during the light phase for per2. The analysis of pcna transcripts revealed a significant rhythmic oscillation, with expression peaks during the light-dark transition. Moreover, the cells were able to re-entrain to a light cycle inversion, showing a completely inverted expression profile of all genes, including pcna, after 3 days under the alternate LD cycle. In addition, under constant LL and DD conditions, rhythmic clock gene expression persisted but with decreasing mesors and amplitudes, especially in the case of per2 and bmal1. These results indicate that seabream-derived cell lines contain a functional molecular clock entrained by light. SAEC-A3 and SAEC-H7 are revealed as valuable cell systems to decipher light-dependent signaling pathways into the vertebrate circadian clock.

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Symposium 10: Telomere, diet and human disease

S10-01

PREDIABETES, DIABETES AND ALZHEIMER'S DISEASE: PRECLINICAL MODELS AND TRANSLATIONAL IMPLICATIONS

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Alzheimer's disease (AD) and vascular dementia are the most common causes of dementia. The borderlines between AD and vascular dementia are also blurred, and while ageing remains the main risk factor to develop AD, epidemiological and clinical studies also support the role of metabolic disease as a significant contributor. To further analyze this complex relationship we have developed different animal models that harbor prediabetes, or type 2 diabetes, and AD. Prediabetes was induced in a classical model of AD (APP/PS1 mouse) by long-term administration of a high-fat diet, resembling a westernized diet. AD-T2D mice were generated by crossing APP/PS1 mice with a T2D model (db/db mice). Brain alterations in prediabetic APP/PS1 mice were worsened when compared with AD mice. Furthermore, a synergistic, more severe effect, was observed when AD and T2D were set together in APP/PS1xdb/db mice. Metabolic alterations were aggravated in AD-T2D mice, suggesting a two-way cross talk between both pathologies. At central level, severe brain atrophy, neuronal simplification and spine loss was detected. Likewise, spontaneous central bleeding, tau hyperphosphorylation and inflammation were increased in APP/PS1xdb/db mice. Interestingly, we observed a shift in amyloid-beta deposition, and more toxic soluble species were favored when AD and T2D are set together. Also, initial in vivo multiphoton assessment of APP/PS1xdb/db mice supports changes in amyloid-beta natural history. Moreover, observed alterations in APP/PS1xdb/db mice result in severe cognitive impairment in young mice (14 weeks). Our data suggest that prediabetic APP and APP/PS1xdb/db mice present a progressive and more severe version of AD pathological features. The fact that metabolic parameters predict many of these alterations, supports the possibility of arresting central complication by controlling metabolic alterations associated with T2D. In this sense, antidiabetic treatments may provide an attractive possibility of limiting central pathology associated to T2D and AD, that may ultimately delay or slow down AD progression.

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S10-02

INFLUENCE OF ADIPOSITY AND DIETARY PATTERN ON TELOMERE LENGTH

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Environmental factors (dietary factors) are involved in the development of obesity and other age-related diseases (Marti and Zalba, *Telomeres, Diet and Human Disease: Advances and Therapeutic Opportunities*, CRP Press, 2017). One mechanism by which dietary factors may influence health outcomes is through its potential effects

on telomere, the repeating DNA–protein structures that protect the ends of linear chromosomes and helps to maintain genome stability. Notably, short telomeres are observed in peripheral blood cells from patients with type 2 diabetes, metabolic syndrome and obesity. This presentation has two parts. Firstly, we will examine the relationship between telomere length and adiposity. Research work from our intervention studies revealed that an intensive weight loss intervention resulted in a lower telomere shortening (García-Calzón et al., Plos One 2014) and also that telomere length could be a potential biomarker for changes in adiposity (García-Calzón et al., Int J Obes (Lond). 2014). Secondly, the influence of food items and dietary pattern on telomere length will be revised (Marti et al, Nutr Hosp 2017). Positive associations between telomere length and adherence to the Mediterranean diet or consumption of vegetables and fruits are reported. Meanwhile, processed meats, cereals, alcohol and sweetened beverages are associated with shorter telomeres. Moreover, our work in the frame of the PREDIMED-Navarra study (García-Calzón et al., Am J Clin Nutr 2015; Clin Nutr 2015; 2016) suggested that diet might play a key role as a determinant of telomere length through pro-inflammatory or anti-inflammatory mechanisms.

Telomere length, obesity, dietary pattern, BMI, food items

S10-03

AGING RELATED-CARDIOVASCULAR DISEASES AND TELOMERE LENGTH

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Despite the now well documented fact that cardiovascular disease, coronary artery disease as well as heart failure, is associated with short telomeres, and that short telomere length predisposes to coronary artery disease, there has been no proof of causality until now. Evidence from progeroid syndromes suggests that severe disruption of DNA repair results in shorter mean telomere length and cellular senescence, which in turn can cause premature atherosclerosis. Short telomere length in peripheral blood leukocytes itself can 1) have an inherited component without the co-existence of DNA damage, 2) indicate increased cell turnover e.g. under systemic low grade inflammation, or 3) be a surrogate for accelerated DNA damage and cell senescence. In order to make more informed conclusions about the causal relationship between telomere length and coronary artery disease, further research is needed, both in clinical studies and on a molecular level. In this talk, we will discuss our work related to telomere length in coronary artery disease, and show a) how telomere length in the bone marrow correlates with peripheral blood telomere length, b) if telomere length shortening in coronary artery disease is across all leukocyte subpopulations, c) how telomere length shortening in CD8 T-cells reflects inflammation, and d) how ageing affects telomere length heterogeneity. Using primary lymphocytes and myeloid cell cultures, we demonstrate that cultivation under hyperoxic conditions induced oxidative stress resulting in chronic activation of CD4+ cells and significantly reduced CD4+ T-cell proliferation. The latter was telomerase dependent because oxidative stress had no effect on the proliferation of primary lymphocytes isolated from telomerase knockout mice. In contrast, myeloid cell proliferation was unaffected by oxidative stress nor reliant on telomerase.

Symposium 11: Mitochondrial physiology reinventing complexity

S11-01

MOLECULAR MECHANISMS OF MITOCHONDRIAL DYSFUNCTION

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(Abstract not available)

S11-02

MITOCHONDRIAL DYNAMIC DURING EXERCISE: FUSION-FISSION AND SUPERCOMPLEXES ASSEMBLY

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A negative aspect of sport practice, is the elevated production of superoxide radical as a consequence of the high oxygen consumption or by xanthine oxidase activity. According to the mitochondrial theory of aging, elite athletes will be more vulnerable because they are lifelong more exposed to deleterious effect of superoxide radical. Surprisingly, and against this theory, we have recently demonstrated in elite athletes, a less lipid peroxidation at rest and after a submaximal exercise. This phenomenon also occurs after training at moderate hypoxia (2320 meters over sea level) that generates less amount of oxidative stress markers with respect to the same intensity and volume training in normoxia environments. This lesser lipid peroxidation cannot be solely justified in base to antioxidant defense mechanisms, enzymatic and nonenzymatic, because they do not change or decreases. In this presentation, we analyze the possible mechanisms involved and probably related to unknown mitochondrial adaptations. More concretely with the mitochondrial respiratory complexes that are known to undergo assemblies into supercomplexes (SCs) under physiological conditions. One of the functional roles of these entities is the limitation of reactive oxygen production through Complex I. In fact, we have verified that a cross-training program (HIT / HVI) induces the Complex I assembly into SCs together with a systemic reduction of lipid peroxidation. We also reported an inverse relationship between Complex I superassembly and mitochondrial lipid peroxidation. We conclude that Complex I assembly into SCs may be a potential mechanism underlying the antioxidant effects of exercise. In this presentation we also analyze the role of mitochondrial fusion-fission dynamics and the gene expression of the key proteins involved in this phenomenon, comparing rtPCR data and electronic microscopy images. This study could provide another point of view on aspects related to mitochondrial bioenergetics efficiency and movement economy.

Mitochondrial, mitochondrial supercomplexes, PGC-1 α , mitofusins 2, FIS1, hypoxia

S11-03

FUNCTION OF MITOCHONDRIAL SUPERCOMPLEXES: COENZYME Q CHANNELLING AND CONTROL OF ROS GENERATION

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It has been known for over 60 years that the respiratory chain of mitochondria is constituted of a series of protein complexes imbedded in the inner mitochondrial membrane, that assure electron transfer from reduced substrates to molecular oxygen. More recently, experimental evidence has ascertained that the major respiratory complexes involved in energy conservation (Complexes I, III and IV) are assembled as supramolecular units (supercomplexes, SCs) in stoichiometric ratios. The functional role of SCs is not yet completely defined, and still open to discussion and controversy. Several lines of evidence favour the concept that electron transfer from Complex I to Complex III operates by channelling of electrons through Coenzyme Q molecules bound to the SC I1III2IVn, in contrast with the previously accepted idea that the transfer of reducing equivalents from Complex I to Complex III occurs via random diffusion of the Coenzyme Q molecules in the lipid bilayer. At difference with Complex I, Complex II does not associate with other mitochondrial complexes. Surprisingly, electron transfer from Complex III to Complex IV seems to operate, at least in mammals, by random diffusion of cytochrome c molecules between the respiratory complexes even if assembled in SCs. Another property provided by the SC assembly is the control of generation of reactive oxygen species (ROS) by Complex I, as shown by the enhancement of ROS generation under conditions dissociating supercomplexes. This property might be important in regulation of signal transduction from mitochondria, in which ROS provide a redox signalling pathway. It is now clear that supercomplex assembly is involved in several physiological and pathological changes.

Symposium 12: Brain stimulation

S12-01

MODULATION OF BRAIN CIRCUITS BY DEEP BRAIN STIMULATION FOR THE TREATMENT OF DEPRESSION

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Major Depressive Disorder is a psychiatric illness regarded as a major cause of disability, with a high worldwide prevalence. Despite the multiple therapeutic approaches currently available to treat depression, including antidepressant drugs, psychotherapy and electroconvulsive therapy (ECT), an important number of patients do not respond satisfactorily to these conventional therapies; this has led to the investigation of alternative therapeutic modalities. Deep brain stimulation (DBS) is one of these new modalities and some clinical studies are showing promising results to treat patients with refractory depression, but the way in which DBS might achieve these effects remains somewhat enigmatic. I will conduct an integrative review and provide new insights about possible underlying mechanisms for DBS-generated antidepressant effects identified in preclinical studies and clinical trials, and to determine which brain target(s) elicited the most promising outcomes considering acute and maintenance treatment of treatment resistant depression.

S12-02

MECHANISMS OF ACTION OF DEEP BRAIN STIMULATION TO MODIFY BRAIN ACTIVITY

J. J. González-Rosa

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Deep brain stimulation (DBS) promises innovative experimental possibilities for clinical psychology and neuroscience as well as new therapeutic and palliative measures in medicine, been remarkably effective for a range of movement disorders, epilepsy, and some types of mental illness that have failed pharmacological and cell transplant therapies. Thus, DBS is clinically effective in improving motor function of essential tremor, Parkinson's disease and primary dystonia and in relieving obsessive-compulsive disorder, being an effective therapy for an increasingly expanding spectrum of neurological diseases and psychiatric conditions. However, the precise mechanisms that underlie the modulation of the symptoms is not fully understood, and has prompted investigations to better understand the therapeutic benefits of DBS in hope of improving patients outcome. Rather than inducing complete local inhibition as originally thought, DBS may partially block some neural activity and reshape firing patterns by activating other depending on electrode location and stimulation parameters. How DBS modulates these abnormal neuronal activity patterns is yet to be determined. Although depolarization block may remain a major mechanism of action of DBS, in addition to inhibiting the activity in the stimulated gray matter and/or involving activation of white matter fibers, release of neurotransmitters and anti-inflammatory mediators may also be involved in the therapeutic effects of DBS in treatment-refractory brain disorders. Several studies have also shown feasible and positive effects for most of these strategies and their potential clinical relevance to modulates normal brain activity and induces behavioural changes for both animal and human models. Understanding the mechanisms of action of DBS as well as how side-effects are generated is critically important if we are to improve current therapies and understand the potential application of these tools for other brain-affected conditions.

S12-03

TRANSCRANIAL APPLICATION OF STATIC MAGNETIC FIELD OVER THE HUMAN CORTEX

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Plasticity may provide the physiological basis for neuropsychiatric treatments and rehabilitation procedures. The neurophysiological techniques that can induce plasticity or simply modulate cortical excitability or produce interference with normal brain activity and behavior are known as neuromodulation techniques. It is possible to obtain these effects by applying electrical currents directly to discrete regions of the nervous system (deep brain stimulation, epidural cortical stimulation, epidural spinal cord stimulation, etc.). These techniques are invasive. On the other hand, a number of techniques can obtain neuromodulatory effects in a non-invasive manner (Non-invasive brain stimulation-NIBS). The transcranial application of magnetic fields or of electric currents can induce LTP and LTD like after-effects in the human cortex. The most used non-invasive neuromodulation techniques are: low (<1Hz) and high (>5Hz) repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS). Recently, it has been reported the possibility to obtain non-invasive neuromodulation using the transcranial application of static magnetic fields (tSMS). The transcranial application of static magnetic field (tSMS) is able to interfere with brain function. An intensity between 120 and 200 mT at cortical level is able to reduce the excitability of the motor cortex. Moreover, tSMS over the visual and sensory cortices boosts

the oscillatory activity of the alpha band and have behavioral consequences. It is effective, extremely cheap and safe. People can be easily trained to apply tSMS. The device is portable and a treatment at the patient's house is easy as patients and/or caregivers can be trained easily to apply tSMS. tSMS is probably the easiest form to manipulate cortical activity in humans in a relatively focal manner. It is effective, extremely cheap and safe. As SMS does not deliver currents to the brain, all the regulatory pathways required to use it in clinical trials are easier and cheaper than the other NIBS techniques. For this reason, we think that translation for “bench to bedside” should be extremely quick.

ORAL COMMUNICATIONS

Oral session 1: Cell & Molecular Physiology

O1-01

EF-HAND DOMAIN FAMILY MEMBER B REGULATES SOCE BY THE MODULATION OF THE DYNAMIC ASSOCIATION OF STIM1 WITH SOCE-ASSOCIATED REGULATORY FACTOR

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Background: Store operated calcium entry (SOCE) is a major mechanism for Ca^{2+} influx required for the activation of a wide range of cellular functions. After depletion of intracellular Ca^{2+} stores, the association of STIM1 (stromal interaction molecule 1) and Orai1 is the pivotal event in SOCE activation. SARAF (SOCE-associated regulatory factor) has been described as a negative modulator of STIM1 function. Upon store depletion SARAF dissociates from STIM1 to allow the STIM1-Orai1 association, followed by STIM1-SARAF re-association and the inactivation of Orai1. However, this mechanism for fine-tuning SOCE is not completely understood since SARAF does not exhibit Ca^{2+} -binding domains. Here, we have investigated the role of EFHB (EF-hand domain family member B) on the dynamic interaction between STIM1 and SARAF. **METHODS:** Protein expression was demonstrated by Western Blotting. Protein-protein interactions were confirmed by immunoprecipitation. Changes in cytosolic Ca^{2+} concentration were determined by calcium imaging using fura-2 AM. NFAT translocation to nucleus was performed by confocal microscopy.

Results: Silencing EFHB expression in HEK-293 and HeLa cells decreased thapsigargin (TG)-induced SOCE. Immunoprecipitation studies revealed that both STIM1-EFHB and Orai1-EFHB interactions are increased in TG-treated HEK-293 cells co-expressing either STIM1 or Orai1 and a EFHB construct lacking the N-terminal 169aa (EFHB Δ 1-169). Consistent with this, TG-evoked STIM1-Orai1 interaction was significantly greater in cells overexpressing EFHB Δ 1-169, while EFHB silencing reduces TG-evoked STIM1-Orai1 interaction. Accordingly, we observed that EFHB silencing reduces TG-induced translocation of NFAT to the nucleus. Our results indicate that STIM1-EFHB interaction reaches a maximum after 30 s of treatment and then decreased, reaching the resting level 120 s after addition of TG in a medium containing 1 mM Ca^{2+} . In dimethyl BAPTA-loaded cells, the maximum STIM1-EFHB interaction was reached after 30 s of stimulation with TG and is sustained for at least 90 s. TG evoked a significant initial decrease in SARAF-STIM1 interaction that reached a minimum after 30 s and then increased, reaching the resting level 120 s after stimulation. The return of SARAF-

STIM1 interaction to the resting level was impaired in dimethyl-BAPTA-loaded cell, thus suggesting that this process is Ca^{2+} -dependent. Consistent with this, we observed that SARAF-STIM1 dissociation was abolished in cells lacking EFHB, which demonstrates that EFHB is essential for this process.

Conclusion: EFHB is a novel protein involved in the regulation of SOCE by the modulation of the dynamic interaction of STIM1 with SARAF.

Supported by MINECO grant BFU2016-74932-C02-01 and Junta de Extremadura-FEDER grants IB16046 and GR18061. EFHB, SARAF, STIM1, Orai1, calcium entry

O1-02

REGULATION OF GLUT12 EXPRESSION AND ACTIVITY IN 3T3-L1 CELLS AND ADIPOSE TISSUE

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The human facilitative glucose transporter GLUT12 was isolated from the breast cancer cell line MCF-7 by its homology with GLUT4, the main insulin-responsive glucose transporter. GLUT12 is expressed in insulin sensitive tissues such as adipose tissue and skeletal muscle. GLUT12 has been postulated as a second insulin-responsive glucose transporter because in skeletal muscle, insulin promotes GLUT12 translocation to the plasma membrane together with GLUT4. In GLUT12-expressing *Xenopus laevis* oocytes, our group demonstrated that GLUT12 has low sugar selectivity, transporting: D-glucose > α -methyl-D-glucose (α MG) > 2-deoxy-D-glucose (2-DOG) > D-galactose > D-fructose, being α MG a classical SGLT substrate, not transported by other GLUTs. The aim of this work was to investigate GLUT12 expression and regulation in adipocytes and their potential role in obesity. 3T3-L1 cells, a well established model of mouse mature adipocytes, and adipose tissue from lean and diet induced obese mice were used. Protein extraction and plasma membrane isolation from 3T3-L1 cells was performed by serial centrifugations. GLUT12, AKT, AMPK, HIF-1 α and β -Actin were detected by Western blot and GLUT12 was also detected by immunohistochemistry assays. Sugar uptake measurements were performed in 3T3-L1 cells using radiolabeled sugars (α MG and 2-DOG). GLUT12 is expressed in homogenate of 3T3-L1 cells and visceral adipose tissue. GLUT12 localization is mainly perinuclear. Uptake of 2-DOG is inhibited by α MG, indicating functional presence of GLUT12 in the membrane. Insulin and TNF- α increase α MG uptake by inducing GLUT12 translocation to the membrane through AKT and AMPK activation respectively. On the contrary, leptin and adiponectin induce GLUT12 internalization decreasing sugar uptake. The same results were obtained in explants of mice adipose tissue. Hypoxia, confirmed by the expression of the hypoxia marker HIF-1 α , upregulates GLUT12 expression in 3T3-L1 cells. In lean mice, intraperitoneal insulin injection increases GLUT12 expression and AKT phosphorylation. In obese mice, GLUT12 expression is decreased compared to lean animals. Moreover, insulin injection does not have any effect on GLUT12 and AKT phosphorylation in these obese animals. Altogether, these results demonstrate the expression, functional activity and regulation of GLUT12 in adipocytes and contribute to the comprehension of GLUT12 physiological role in adipose tissue and obesity.

GLUT12, adipocytes, TNF- α , insulin, adipokines, obesity

O1-03

SIGMA-2 RECEPTOR IS AN INHIBITOR OF PROLIFERATION AND MIGRATION IN TRIPLE-NEGATIVE BREAST CANCER CELLS, MDA-MB-231 CELLS

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Sigma-2 receptor (σ 2R) is overexpressed in certain cancer cells [1-2]. Our results indicate that treatment of the triple negative breast cancer cell line MDA-MB-231 with the σ 2R-antagonist (SM-21; 100nM) increased cell proliferation after 48h. In addition, treatment of these cells with the dual σ 2R/ σ 1R-agonist (PB28, 10 nM), also enhanced cell proliferation itself, observing differences since the initial 24h. But, cell treatment with PB28 together with SM21 resulted in a similar proliferation pattern than PB28 alone, thus indicating that PB28 alters cells proliferation mainly through σ 1R. Additionally, SM21 enhanced MDA-MB-231 cells migration, while the fluorescent σ 2R-agonist (NO1 100 nM) slow-down the migration ability of these cells. Finally, incubation of MDA-MB-231 cells with NO1 resulted in an increase of the apoptosis rate as estimated by analysing the incorporation of BrdU. NO1-evoked apoptosis resulted in a similar extent than 50 μ M of the antineoplastic drug, cisplatin; while neither cisplatin nor NO1 evoked significant changes in the percentage of apoptosis of the non-tumoral cell line MFC-10A. Interestingly, no localization of NO1 into mitochondria was observed by using confocal microscopy; therefore, we could exclude the activation of a mitochondrial-dependent apoptotic pathway. Altogether, our results indicate that σ 2R is an inhibitor of cell proliferation and migration probably by inducing apoptosis in triple negative breast cancer cells, which aggress with previous observations done using other types of breast cancer lines, like MCF-7 [3].

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Sigma2 receptor, SOCE, cancer

O1-04

PROGESTERONE EVOKES INTRACELLULAR CALCIUM MOBILIZATION THROUGH THE ACTIVATION OF THE PROGESTERONE RECEPTOR MEMBRANE COMPONENT 1 (PGRMC1) IN TRIPLE-NEGATIVE BREAST CANCER MDA-MB-231 CELLS

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Triple negative breast cancer cells (TNBCC) lack the classic intracellular estrogen and progesterone receptors, which confers resilience to the common hormone therapies. In TNBCC lines, such as the MDA-MB-231 cells, some groups have described the existence of a novel Progesterone Receptor Membrane Component 1 (PGRMC1), that allows these cells to respond to progesterone [2]. Here, we aim to elucidate the intracellular pathways downstream of the PGRMC1. In MDA-MB-231 cells Progesterone (1 μ M) stimulation resulted in a steady calcium release from the intracellular calcium stores, as well as calcium entry. Incubation of MDA-MB-231 cells with the σ 2 receptor antagonist, SM21 (100 nM), did not reduce the

calcium mobilization evoked by progesterone in these cells, despite previous studies indicate that both receptors respond to this hormone. In addition, using siRNA against the PGRMC1, we were able to abolish the progesterone-evoked calcium release and calcium entry. Finally, in agreement with previous studies, we observed that progesterone impairs MDA-MB-231 cell proliferation during the initial 48 h, while slightly reduced the proliferation rate in cells lacking PGRMC1. Altogether, our data support a relevant role of PGRMC1 in the biology of TNBCC which might be used as a possible target for anticancer therapies.

Supported by MEC (BFU-2016-74932-C2-1-P) and Junta de Extremadura (GR18061 and IB16046). C Cantonero was granted with a predoctoral fellowship from the Junta de Extremadura. [1] Santana-Davila R et al. 2010. *J Hematol Oncol.*, 27:42. [2] Clark NC et al. 2016. *Cancer Biology and Therapy*, 17:262-271.

MDA-MB-231, progesterone, PGRMC1, calcium, proliferation

O1-05

MELATONIN AS ALTERNATIVE TO HSP90 INHIBITION-INDUCED CHEMORESISTANCE IN U937 LEUKAEMIA CELLS

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HSP90 is constitutively expressed in normal conditions, its role is essential for cell protection and survival. Although, when the protein functionality is threatened by stressing environment, in order to prevent the unfolding or misfolding of proteins, HSP90 is upregulated as part of “stress response”. When stressor disappears the levels of HSP90 diminishes to normal levels and the cell physiology continues normally. Mild stress or exogenous HSP90 are known to be responsible of increased HSP levels, which are concomitant with apoptosis resistance. Molecular alterations that cannot be addressed by the stress response cause lethal stress which leads the cell to the apoptotic death. On the other hand, timely extended stressing environment entails overexpression of HSP90 which is closely linked with the acquirement of stress tolerance, and represents the ideal conditions prone to oncogenesis. Actually, HSP90 is highly expressed in leukaemia cells, and its inhibition has been targeted in leukaemia as a promising strategy, however, cases of resistance to this treatment still remain. Combinations of HSP90 inhibition (tanespimycin, 17-AAG) with other anticancer treatments were considered, although, unfortunately the results were not that synergistic as expected. Our results have shown melatonin as a smart molecule with dual capacity to induce apoptosis in tumoral cells and to protect healthy cells. Additionally, melatonin has been used in combination with other drugs with encouraging outcomes in cancer cells. In this study, we aim to analyse the anti-leukaemia effects of anti-cancer drugs (5-fluorouracil, etoposide, cytarabine) or melatonin in the leukaemia U937 cell line under HSP90 inhibition (17AAG, 24 h). After 24 h of HSP90 inhibition, U937 cells presented an oncostatic response, causing their growth arrest (Neubauer cell counting). The surviving cells show a particular apoptosis resistance (MTT assay, nuclear morphology) when U937 cells were treated with DNA Damaging anti-cancer drugs. Alternatively to anti-cancer drugs, melatonin was much more efficient inducing apoptosis in surviving cells. In this study, we show that HSP90 inhibition could cause drug resistance that might be successfully addressed by melatonin. It is widely known the protection capacity of melatonin in non-tumour cells under toxic agents.

Therefore, the combination of HSP90 inhibition and melatonin could offer oncogenic and pro-apoptotic effect in leukaemia cells, and likely reducing the side effects.

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Apoptosis resistance, HSP90, melatonin, chemosensitivity

O1-06

TRASIENT RECEPTOR POTENTIAL CANONICAL 6 CHANNELS MODULATE STORE-OPERATED CALCIUM ENTRY IN BREAST CANCER CELLS

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Store-operated calcium entry (SOCE) is the major pathway that regulates intracellular Ca^{2+} homeostasis in non-excitable cells [1]. Alterations in SOCE have been associated to a number of disorders including cancer [2]. Breast cancer cells express the key proteins that mediate SOCE: STIM, Orai and TRPC; however, their expression and function varies from ER+ to triple negative cancer cells. By using molecular biology, biochemistry and fluorescence imaging microscopy techniques we have found that the TRPC6 channel, which is overexpressed in the ER+ MCF7 (where Orai3 expression is also enhanced) and triple negative MDA-MB-231 (being Orai1 the predominant channel) breast cancer cell lines, associates with both Orai1 and Orai3 in resting and upon intracellular Ca^{2+} store depletion. In addition, we have found that TRPC6 knockdown impairs SOCE leading to a dramatic inhibition of migration and invasion in the breast cancer cell lines. Altogether, our results show a role for TRPC6 in the regulation of SOCE, migration and invasion in breast cancer cells. Supported by MINECO (BFU2016-74932-C2-1-P) and Junta de Extremadura-FEDER (IB16046 and GR18061). IJ and RD-B are supported by Juan de la Cierva IJCI-2015-25665 and Grant BFU2016-74932-C2-1-P, respectively. 1.Putney, J.W., et al., *Biochim Biophys Acta* (2017) 1864: 900-906. 2.Jardín, I. and Rosado, J.A., *Biochim Biophys Acta* (2016) 1863: 1418-1426.

SOCE, TRP/Orai channels, Breast Cancer

Oral session 2: Sports & Pregnancy

O2-01

EFFECTS OF EXERCISE AND DIET ON BLOOD PRESSURE AND ABDOMINAL FAT IN OBESE RATS AT AN EARLY AGE

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Major consequences of being overweight or obese include higher prevalence of hypertension and a cascade of associated cardiorenal and metabolic disorders. Physical activity is considered a cornerstone for the treatment for obesity because exercise reduces adiposity. The main objective of this study was to evaluate the effects of exercise on addition to the change of diet in

blood pressure (BP), renal hemodynamic and abdominal fat (AF) in obese rats from an early age. In addition, leptin is an adipokine that has an important role in the regulation of BP in obesity, therefore, it will also be analyzed the involvement leptin in the BP levels in obese rats with and without exercise. The animals were randomly divided into eight groups: male and female rats with normal or high fat (HF) diet from weaning. At 4 months of age, the obese groups switched to a standard diet and the corresponding experimental groups began the training program for two months. BW, AF (determined by computed tomography), BP (measured using tail cuff plethysmography), plasma leptin and glomerular filtration rate (GFR) were evaluated before and after exercise protocol. BP decreased ($P<0.05$) in male (149 ± 3 to 133 ± 4 mmHg) and female (127 ± 3 to 111 ± 4 mmHg) obese rats subjected to exercise for two months. Not significant changes in BP were found in the rest of experimental groups. Leptin concentration decreased ($P<0.05$) in response to exercise in male (7.268 ± 502 to 3.799 ± 984 pg/ml) and female (4.232 ± 157 to 2.287 ± 469 pg/ml) rats that previously had a HF diet. The rest of the experimental groups did not show significant changes. AF decreased ($P<0.05$) by 20% in male and 13% in female obese rats that were subjected to exercise. No significant changes were found in the rest of the groups with or without exercise. Body weight increased in all groups without exercise, as did the groups subject to training, except obese males rats who maintained their body weight. No significant changes of GFR were found in any experimental groups with or without exercise. The effects of high fat diet does not disappear if only the diet is modified. Exercise, along with diet, reduces abdominal fat and blood pressure in obese rats. Also, the increase in plasma leptin levels can mediate hypertension in obese rats.

Exercise, leptin, obesity, fat, hypertension

O2-02

HUMAN MUSCULAR MITOCHONDRIAL FUSION DURING EXERCISE

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Skeletal muscle mitochondria form complex networks which are structurally regulated by fusion and fission processes. It is well-known that mitochondrial fusion is a key process in order to maintain the energetic demands of the cell. Indeed, an enlargement of the mitochondrial reticulum has been observed after long-term exercise. However, whether mitochondrial fusion can be induced during intense exercise is unknown. Hence, we investigated the hypothesis that exercise will induce mitochondrial fusion. Well-trained swimmers ($n=9$) performed a duration-matched sprint interval training (SIT) and high-intensity high-volume training (HIHVT) programme on separate days. Muscle samples from M.triceps brachii were taken before, immediately after and 3 hours after the training sessions. Transmission electron microscopy (TEM) was applied to assess mitochondrial morphology. Moreover, expression of genes coding for regulators of mitochondrial fusion and fission were assessed by qRT-PCR. In addition, mitofusin 2 (MFN2) was quantified by Western blot analysis. TEM analyses showed that SIT increased the branching complexity of

intermyofibrillar (IMF) mitochondria only in the transversal plane, although from HIHVT both IMF and subsarcolemmal (SS) increased their branched complexity. After, we analysed markers of mitochondrial size (area and perimeter). The results showed that from both SIT and HIHVT mitochondrial raised their area and perimeter. In response to HIHVT, there was a strong change towards a greater area and perimeter from a transversal plane than from a longitudinal plane. Then, we analysed quantify glycogen (mean/area) and found that IMF glycogen content decreased post SIT but not from HIHVT. However, SS glycogen content decreased from both SIT and HIHVT post training and, at 3 h post training, glycogen began to increase only in HIHVT. An analysis of mRNA levels of mitofusin 1 and 2 (MFN1, MFN 2) showed that both were induced post training in response to SIT, but not from HIHVT. Finally, we found that MFN 2 protein expression was increased only at 3 h in response to both HIHVT and SIT. In conclusion, mitochondrial fusion can be induced during intermittent intense exercise. We suggest that the two exercise protocols applied may differ in the physiological mechanism that triggers mitochondrial fusion.

Mitochondria, Skeletal muscle, Exercise, Mitofusin

O2-03

EFFECTS OF PHYSICAL EXERCISE AND INTAKE OF HYDROXYTYROSOL IN THE REDOX STATUS OF EXERCISED WISTAR RATS

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Purpose: Polyphenols function as antioxidants due to their chemical structure composed of several hydroxyl groups on aromatic rings. Hydroxytyrosol (HT) is the main phenolic compound of olive oil, responsible for its beneficial effects on health and one of the component most biologically active and metabolized by humans. Accordingly, among other biological properties, HT has powerful *in vivo* antioxidant effects (HU, T. et al. 2014; Manach, C. et al. 2004). The aim of our study is to describe the effects of different doses of HT on REDOX capacity in sedentary and exercised rats. **Methods:** 40 male Wistar rats were distributed in 6 groups: Sedentary (Sed), SED with intake of 20mg/kg/day of HT (SED20), SED with intake of 300 mg/Kg/day of HT (SED300), Exercised (EXE), EXE with 20 mg/Kg/day of HT, EXE with 300 mg/Kg/day of HT. During 20 weeks of experimental project; the maximum running speed (3 tests during the study) and the daily work was evaluated. Hemoglobin (HGB), and Hematocrit (HCT) were measured in blood. Finally, the concentration of mitochondrial plasma hydroperoxides as a marker of lipid peroxidation (oxidative stress) was evaluated. **Results:** In Sedentary rats HT induced an antioxidant effect without implying improvements in sports performance. However, in combination with the exercise the dose of 300 mg/kg/day produced a pro-oxidant effect. **Conclusion:** In summary, HT dosages ranging from 20 mg/kg/d to 300 mg/kg/d for 10 weeks induced an antioxidant response in a dose-dependent manner in sedentary animals. However, 20 mg/kg/d HT decreased the running capacity when this dose was supplemented during exercise, whereas 300 mg/kg/d HT was able to maintain and even increase the running capacity. This effect might be due to a systemic pro-oxidant effect induced when a high HT dose is supplemented during exercise training (Boots, AW. et al. 2007). **Future studies:** We evaluate the results related to mitochondrial biogenesis (Supercomplexes), and we are currently beginning to analyze the genetic manifestations that may be involved in our project.

Polyphenols, ROS, exercise, Oxidative stress, antioxidants

O2-04

POTENTIAL BIOMARKERS IN EARLY PREGNANCY TO PREDICT MATERNAL COMPLICATIONS

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In the last decades there has been a rise in the age of first pregnancy. Maternal ageing is associated with an increase in obstetric complications, being particularly important gestational hypertension (GHT), pre-eclampsia (PE) and gestational Diabetes Mellitus (GDM). These situations compromise materno-fetal health and program the offspring for cardiometabolic disease development. Therefore, it is important to detect women at risk in early pregnancy. GHT, PE and GDM are associated with oxidative stress, inflammation and alterations in angiogenesis. Several potential biomarkers have been proposed but they show high interindividual variability and have been detected after the complication is established. We hypothesize that a poor maternal antioxidant status in early pregnancy may associate with later development of obstetric complications and could be used as biomarker.

Objectives. 1) To assess several plasma antioxidants in early pregnancy and to calculate a score indicative of global maternal antioxidant status (Antiox-S); 2) to evaluate the association of Antiox-S with development of obstetric complications; 3) To analyze several cytokines implicated in inflammation and angiogenesis.

Methods. 200 healthy women (34.4 years old) were recruited at University Hospital La Paz (Spain). At week 9 of pregnancy a blood sample was obtained and the women were followed-up until labor, analyzing if they developed a normal pregnancy (N) or maternal complications (MC). Plasma was used to evaluate the following antioxidants: superoxide dismutase and catalase activities, GSH, thiol groups and phenolic compounds by spectrophotometry. Antiox-S was calculated averaging these antioxidants after a normalization and typification process. The antioxidant hormone Melatonin and the following cytokines (TNF- α , MCP-1, IL-1 β , IL-8) were assessed by competitive and indirect ELISA, respectively.

Results. 39.5% of the women developed minor MC; 11% developed severe MC (GHT=2.2%, PE=4.9%, GDM=6%). In comparison with normal pregnancies women who developed a complication exhibited at week 9 a significantly lower: 1) Antiox-S (MC=-0.07+/-0.05; N=0.07+/-0.04 arbitrary units); 2) melatonin levels (MC=2.4+/-0.1pg/ml; N=2.8+/-0.1pg/ml). IL-8 was significantly lower only in women who developed PE (PE=1.5+/-0.8 pg/ml; N=5.5+/-0.9 pg/ml).

Conclusions. A low global antioxidant status in early pregnancy associates with the development of a maternal complication. This antioxidant score could be considered as potential predictive biomarker. A lower level of IL-8 in women who develop PE may be related to a poor angiogenesis and impaired spiral artery remodelling. These parameters should be analyzed in a broader population to confirm their validity as biomarkers.

Biomarkers, Obstetric complications, Antioxidants

O2-05

ASSESSMENT OF PHYSIOLOGIC CHANGES DURING PREGNANCY USING NON-INVASIVE METHODS

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Uterine, renal and systemic hemodynamic changes during pregnancy have been extensively examined. However, the evolution of these hemodynamic changes have not been evaluated in the same subject. The main objective of this study was to examine the simultaneous evolution of uterine, renal and systemic hemodynamic changes during pregnancy in the same subject, by using new non-invasive and highly reproducible methods. This study was conducted in female rats (n=12) during nonpregnancy period and at days 7, 14 and 19 of gestation and the authors analysed systolic arterial pressure, glomerular filtration rate (GFR), cardiac output, uterine and renal blood flow. The results presented are considered novel and interesting for the monitoring of pregnancy parameters by using noninvasive, sensitive, and reproducible method. Arterial pressure decreased ($P < 0.05$) from 108 ± 2 mmHg before pregnancy to 99 ± 3 mmHg during the second and third week of pregnancy. GFR increased ($P < 0.05$) the first (26%) and second (28%) week but the increment was greater ($P < 0.05$) at the end of pregnancy (63%). RBF only increased transiently during midgestation (10.1 ± 0.5 ml/min vs 8.3 ± 0.5 ml/min during the nonpregnancy period, $p < 0.05$). Cardiac output increased ($P < 0.05$) during the first (28.5%), second (29.8%) and third (45.7%) week of pregnancy but heart rate was only elevated at midgestation (8.9%, $P < 0.05$). Peak systolic velocity (PSV), mean velocity and velocity time integral (VTI) increased progressively in the main uterine artery and its secondary branches through gestation. In the main uterine artery, PSV was 403 ± 34 , 596 ± 24 and 705 ± 27 mm/s at days 7, 14 and 19 of gestation, respectively. VTI was 36 ± 3 , 61 ± 3 , 72 ± 3 mm at days 7, 14 and 19 of gestation, respectively. These results demonstrate that Doppler ultrasonography is a non-invasive method that can be useful to detect long-term physiologic changes during pregnancy, and that the sinistrin clearance is a highly reproducible method to examine progressive GFR changes in conscious rats. These data are benchmarked against the conventional methods to examine renal, systemic and uterine hemodynamic changes demonstrating that small animal sonography is a noninvasive, sensitive, and reproducible method, which has minimal requirement of animal use. Thus, it is an effective tool for early detection of changes in physiologic and pathologic situations using rat models.

Pregnancy, ultrasonography, renal, uterine, flow

O2-06

TWENTY FOUR HOURS OF EXPOSURE TO MODERATE HYPOXIA DOES NOT INCREASE THE ASSEMBLY OF MITOCHONDRIAL SUPERCOMPLEXES

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Background: Recent findings from our research group demonstrated a systemic reduction of the oxidative stress in swimmer at moderate hypoxia, as denoted by a remarkable decrease in lipid peroxidation. This improve has been partially explained by a tocopherol mobilization, however, it could be also due to an increase of the assembly of mitochondrial supercomplexes. **Aim:** The purpose of the present study was to evaluate whether exposure to moderate hypoxia increases the assembly of mitochondrial supercomplexes in a rats.

Methods: The normoxia situation was performed at the Biomedical Research Centre of University of Granada, at 630 metres above sea level. The hypoxia situation was performed at the High Performance Altitude Training Centre of Sierra Nevada (CARD) located in Granada 2320 metres 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=6), 6 hours group (n=6) and 24 hours group (n=6). 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. Statistical comparisons were determined using an ANOVA analysis of variance. The level of significance was set at $p < 0.05$.

Results: Neither complex I nor complex III showed a significant assembly into supercomplexes ($p > 0.05$). Nevertheless, the assembly into supercomplexes of mitochondrial complex III after 6h of moderate hypoxia exposure increased 14% if compared with normoxia.

Conclusion: Acute exposure to moderate hypoxia does not increase the assembly of mitochondrial complexes I and III into supercomplexes.

Moderate hypoxia, Complex I, Complex III

Oral session 3: Cardiovascular I

O3-01

UROCORTIN-2 PREVENTS DYSREGULATION OF Ca^{2+} HOMEOSTASIS AND IMPROVES EARLY CARDIAC REMODELING AFTER ISCHEMIA AND REPERFUSION

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Background information: An early revascularization successfully limits the extent of myocardial damage in patients with heart infarcts. However, revascularization might cause cardiac injury what is known as Ischemia and Reperfusion (I/R) syndrome, leading to the adverse cardiac remodeling and heart failure. Therefore, cardioprotection strategies still remains of major interest. Cardiac remodeling is a multifactorial process involving fibrosis, hypertrophy and alteration in the expression of genes and proteins' expression related to Ca^{2+} homeostasis such as Transient Receptor Potential Canonical (TRPC) or Store Operated Ca^{2+} Channels (SOCC). **Aim:** We aim to examine the effect of Urocortin-2 (Ucn-2), a potent cardioprotector, against I/R injuries focusing on Ucn-2 regulation of $[Ca^{2+}]_i$ handling.

Methods: Wistar rat model of I/R was induced by transient ligation of the left coronary artery. In other experimental treated group Ucn-2 was intravenously administrated 5 minutes before reperfusion. The study of $[Ca^{2+}]_i$ transients was performed in isolated adult cardiomyocytes using confocal imaging. Meanwhile, the role of Store-Operated Ca^{2+} Entry (SOCE) was examined in Neonatal Rat Ventricular Myocytes (NRVM) submitted to simulate in vitro I/R protocol.

Results: The results indicate that Ucn-2 improves heart's performances, it recovers the ejection fraction and fractional shortening, which are decreased in I/R's experimental group. Ucn-2 also improves other macroscopic parameters as it decreases the infarct size and attenuates fibrosis. Furthermore, Ucn-2 prevents I/R dysregulation of $[Ca^{2+}]_i$ handling in

isolated cardiomyocytes because it improves cell shortening and recovers the amplitude of $[Ca^{2+}]_i$ transients as compare to cells from I/R's rats. Interestingly, the $[Ca^{2+}]_i$ dysregulation was associated with changes in the expression of genes related to Ca^{2+} homeostasis, such as TRPC channels, Orai1/2 and STIM1/2. Therefore, we validated the expression of these genes and we explored their possible regulation by the infusion of Ucn-2 in I/R's rats. Using NRVM, we first determined that silencing of Orai1 and TRPC5 inhibits I/R-induced exacerbated SOCE. Second, we observed that Ucn-2 downregulates the expression of Orai1 and TRPC5 and inhibits I/R induced SOCE. Finally and using proximity ligation assay, we demonstrated that Ucn-2 attenuates the interaction between TRPC5 and Orai1 channels under I/R.

Conclusions: Our study provides the first evidences demonstrating that Ucn-2 addition at the onset of reperfusion attenuates I/R-induced adverse cardiac remodeling, involving the handling of $[Ca^{2+}]_i$ and the inhibition of TRPC5 and orai1 expression and interaction.

Urocortin-2, TRPC5, Orai, SOCE, Ca^{2+} , ischemia

O3-02

ANALYSIS OF PRO-INFLAMMATORY MONOCYTE SUBSETS IN VENTRICULAR ADVERSE REMODELING. IMPLICATIONS IN HEART FAILURE

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Introduction: Primary percutaneous transluminal coronary angioplasty (PTCA) is nowadays consider the best therapy to improve survival of patient with STEMI (ST-Elevation Myocardial Infarction). Nevertheless, despite successful revascularization procedures different structural and molecular alterations have been observed in the myocardium which trigger ventricular adverse remodeling. This remodeling occurs due to several process involving inflammatory cell infiltration in the myocardium. Emerging clinical analysis revealed a higher prevalence of monocytosis in cardiovascular diseases. Moreover monocyte count is increased in acute myocardial infarct (AMI) patients compared to patients with stable coronary arterial disease. This study aims to analyze monocyte subsets in STEMI patients undergoing PCTA and their possible correlation with ventricular adverse remodeling.

Methods: The main goal of this study is to quantify the level of monocytes subsets and correlate them in patients who develop ventricular adverse remodeling after an AMI. We studied 35 STEMI patients who underwent a PTCA with the following clinics: patients with no cardiovascular event records, with an anterior descendent coronary artery occlusion. We extracted peripheral blood before PCTA and 6-12 hours after the reperfusion procedure.

Results: We analyzed 3 monocyte subsets based on the cell surface expression of CD14 and CD16. CD14⁺⁺CD16⁻ (classical monocytes), are the most prevalent subset in human blood. The CD14⁺⁺CD16⁺ (intermediate monocytes) which are the most pro-inflammatory subset and the CD14⁺CD16⁺⁺ (non-classical monocytes) which perform an in vivo patrolling function. Subsequently we performed an echocardiography study 6 months after the surgical procedure to detect patients who exhibit ventricular adverse remodeling. We observed that the level of intermediate and

non-classical monocytes was higher in patients who underwent ventricular remodeling.

Conclusions: The preliminary results suggest that the level of these monocyte subsets will allow us to find and validate potential cell population candidates involved in the adverse remodeling.

Cardiovascular pathophysiology, Myocardial infarction, Heart failure

O3-03

IMPLICATION OF OXIDATIVE STRESS AND RENIN ANGIOTENSIN SYSTEM IN THE VENTRICULAR HYPERTROPHY IN A RAT MODEL OF FETAL PROGRAMMING OF HYPERTENSION (SPRAGUE DAWLEY RATS)

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Background. Fetal undernutrition is associated with the development of hypertension and heart disease, a process known as fetal programming. In a rat model fetal programming, induced by maternal undernutrition (MUN) we have evidence of left ventricular hypertrophy (LVH)⁽¹⁾ and plasma oxidative damage⁽²⁾ in male offspring at weaning. Among the responsible mechanisms, oxidative stress alterations, renin-angiotensin system or high blood pressure may play a role. **Objectives.** To assess if early LVH is related to alterations in angiotensin II receptors, cardiac oxidative status or hypertension.

Material and Methods. 21-day old male offspring from rats fed ad libitum (control) or with 50% of the normal daily intake during the second half of gestation (MUN) were used. Intra-arterial blood pressure was measured in anesthetized rats. The ventricles were removed, weighted and processed for immunohistochemical analysis or Western Blot. Ventricle sections were incubated with Sirius Red and interstitial collagen was detected with polarized light. AT1, AT2, Mas and MrgD receptor density was assessed by immunohistochemical analysis followed quantitative analysis with PAQI software (developed by Porto University). p22phox, SOD1, SOD2, Ec-SOD and catalase protein expression was evaluated by Western Blot, using GADPH as charge control. **Results.** We did not detect statistical differences in blood pressure or heart rate between MUN and control rats. MUN rats exhibited a significantly higher ventricle weight/body weight and heart interstitial collagen content compared to control rats. The 4 types of receptors studied were detected in cardiac tissue, being AT2 and MrgD immunoreactivity lower than AT1 o Mas. AT2 y MrgD immunoreactivity was lower in MUN compared to control rats. p22phox protein expression was significantly higher in MUN compared to control rats and no significant differences between groups were detected in any of the antioxidant enzymes studied. **Conclusions.** Fetal undernutrition induces LVH and fibrosis, probably due to excessive production of free radicals by NADPH-oxidase, with the implication of an imbalance between angiotensin II receptor subtypes.

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Fetal programming, angiotensin II receptors, hypertension

O3-04

DEVELOPMENT OF A MODEL OF CHRONIC INFARCTION (SIX WEEKS) IN RABBITS TO INVESTIGATE THE INDUCIBILITY OF REENTRANT ARRHYTHMIAS

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Introduction: Ischemic heart disease is a leading cause of death in industrialized world. In myocardial damage due to ischemia/reperfusion, there are processes that develop ventricular dysfunction, heart failure, ventricular arrhythmias that produce sudden death. One of the possible arrhythmogenic deleterious processes is the fibrotic response due to cardiac remodeling. Purpose: we present a model of chronic infarction in rabbits for the analysis of inducibility of ventricular fibrillation (VF) and/or sustained ventricular tachycardia (VT), as a previous step to further investigations to elucidate the arrhythmogenic role of fibrosis.

Material and methods: 28 male White New Zealand rabbits were divided into two groups: sham operated group (SO) and chronic myocardial infarction group (MI). The animals were pre-anesthetized with ketamine 20 mg and xylazine 100 mg i.m. and premedicated with an analgesic, anti-inflammatory and antibiotic. Animals were anesthetized intravenously with propofol 10 mg/ml. Once anesthetized, endotracheal intubation was carried out. After intubation, the animals remained mechanically ventilated and anesthetized by inhalation with isoflurane. Medium sternotomy and pericardiotomy were performed. The circumflex artery was occluded halfway through its epicardial tract, by transfixion. The occlusion (1 hour) was followed by reperfusion. In SO, the coronary artery was not occluded. Once the procedure was finished the chest was closed according to standard procedures. Post-operative care: analgesia, initial parenteral nutrition, antibiotic therapy and anti-inflammatories. After 5 weeks animals were sacrificed, the heart was removed, isolated and perfused according to the Langendorff technique to analyse the inducibility of VF by applying the ventricular extrastimulus test with 2 different stimulation cycles (250 and 150 ms) and in four peripheral zones to the infarct (recorded with a multi-electrode plate). A chi-square test was applied to analyse the inducibility of arrhythmias.

Results: Six weeks after the infarction production, we found areas of infarction identified with macroscopic staining techniques (triphenyl tetrazolium), within the area at risk, determined by thioflavin staining. No infarct areas appear in SO group. In 10 hearts of 18, from the infarcted group, VF was induced and only two SO of 10, fibrillated ($p = 0.06$). In 14 hearts of 18, from the infarcted group, sustained VF or VT was induced and only three SO hearts (10 cases) exhibited VT or VF ($p < 0.05$). **Conclusion:** A model of chronic infarction in rabbits is validated, useful for studies of arrhythmogenicity under different manipulations. The presence of chronic infarction may explain the tendency to an easier VF and VT induction.

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Electrophysiology, myocardial infarction, arrhythmias, ventricular fibrillation

O3-05

NEW BIOMARKER OF OXIDATIVE STRESS FOR DETECTION OF LEFT VENTRICULAR HYPERTROPHY AND FUTURE THERAPEUTIC TARGET

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Background and Aims: Left ventricular hypertrophy (LVH) is a risk factor for cardiovascular death. An increase in oxidative stress in LVH is well recognised. Our research group investigates new therapies in the early regression of cardiovascular remodeling that act on biomarkers of oxidative stress⁽¹⁾. The aim of the study is to search new biomarkers for the diagnosis of LVH and future therapeutic target.

Methods: Observational, prospective, non-randomized, comparative study of two groups of patients: LVH group ($n = 35$) and control group (without LVH, $n = 35$). The echocardiographic study allowed the detection of patients with LVH. In each patient we performed the extraction of a venous blood sample for the study of oxidative stress markers: total thiols, thiolated proteins and thiolated protein index (TPI = thiolated proteins / thiols). The area under the curve for PTI was calculated and we use the logist regression analysis to estimate the association between PTI and clinical variables of interest. The biomarkers were compared in both group using student independent t-test. All procedures were approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Results: No significant differences were observed between both groups in the total thiols. However, we detected an increase in the thiolated proteins ($P < 0.01$) and PTI ($P < 0.001$) in the HVI Group with respect to the Control Group. The area under the ROC curve for PTI was of 0.75 (95% CI: 0.63-0.86). The regression model demonstrated that PTI is an independent risk factor ($P = 0.02$, OR = 7.68, 95% CI: 1.37- 42.99) for clinical variables (sex, age, arterial hypertension, diabetes mellitus, dyslipidemia, renal insufficiency, coronary/valvular pathology).

Conclusion: In this study we propose a new biomarker of oxidative stress in patients with LVH, the PTI.

Acknowledgements: Study financed by the Health Research Fund FIS 16/02069 and Fondos FEDER. References: ⁽¹⁾ Quintana-Villamandos B, Goukassian DA, Sasi SP, Delgado-Baeza E. *Biomed Res Int.* 2018;2018:2691014.

Ventricular hypertrophy, Oxidative stress, PTI

O3-06

STUDY OF THE INTRINSIC ELECTROPHYSIOLOGICAL STABILITY OF THE VENTRICULAR MYOCARDIUM IN A MODEL OF CHRONIC MYOCARDIAL INFARCTION IN RABBITS

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Introduction/Purpose. In the present work it has been investigated the intrinsic myocardial electrophysiological stability and heterogeneity of the rabbit ventricular myocardium submitted to a model of chronic (six weeks) myocardial infarction. The electrophysiological parameters investigated, in an isolated, perfused and fibrillating heart from rabbits who had a chronic infarction, were the mean dominant frequency (DF) of ventricular fibrillation (VF) and the spectrum concentration (SC) of the signal (see Methods). The variation of these parameters over time was analyzed as a measure of the electrical stability of the myocardium.

Methods. 17 male White New Zealand rabbits were divided into two groups: 7 sham operated animals (SO) and 10 chronic myocardial infarction animals (MI). After pre-anaesthesia and analgesic, anti-inflammatory and antibiotic premedication, animals were anesthetized intravenously with propofol 10 mg/ml. Animals remained mechanically ventilated and anesthetized by inhalation with isoflurane. Medium sternotomy and pericardiotomy were performed and the circumflex artery was occluded (1 hour) halfway through its epicardial tract, by transfixion. The occlusion was followed by reperfusion. In SO, the coronary artery was not occluded. The chest was closed according to standard procedures. Post-operative care were applied. After 5 weeks the animals were sacrificed, the heart was removed, isolated and perfused according to the Langendorff technique to analyse. A pacing electrode and a plaque with 256 recording electrodes were positioned on the left ventricle. VF was induced by pacing, maintaining the perfusion. The DF of VF was obtained by a spectral analysis. The SC (percentage of the total power spectrum, contained in a range of ± 1 hertz around the dominant frequency) as an index of the signal regularity was also determined. The parameters were determined 1, 2, 3 and 4 minutes after the beginning of the VF. An Anova test, repeated measures, was applied.

Results. In the MI group, after 1,2,3 and 4 minutes after the onset of VF, DF decreased over time ($13,4\pm 1,2$, $13,2\pm 1,4$, $13,1\pm 1,3$ and $12,6\pm 1,5^*$, respectively; $n=10$, $*p<0,05$ vs min 1). SC (in percentage) did not change over time in the SO group and in MI group, although in this last group some variations were observed but did not reached statistical significance ($25,3\pm 5$, $26,4\pm 5,6$, $25\pm 5,6$ and $26,6\pm 5,6$; $n=7$).

Conclusion. In our model of chronic infarction in rabbits, electrophysiological instability is produced. It confers to this model, utility for its use in the study of several types of manipulations on arrhythmogenic myocardial parameters.

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Electrophysiology, myocardial infarction, arrhythmias, ventricular fibrillation

Oral session 4: Neurophysiology & Optogenetic

O4-01

STATIC MAGNETIC FIELD STIMULATION REDUCES EPILEPTIC ACTIVITY IN THE ANAESTHETIZED RAT AND MONKEY

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Objective Epilepsy is a neurological disorder that affects 75 million people around the world. It is a complex pathology, with different etiologies (genetic, metabolic defects, brain tumors, stroke, infections, trauma, etc) characterized by recurrent spontaneous seizures, during which abnormal activity is detected at different cortical areas. The aim of this work was to study the effect of Static Magnetic Fields (SMF) on epileptic cortical activity, since SMF have a well-documented capability for reducing cortical activity in both human and animal models.

Methods A total of 14 Sprague-Dawley rats and 1 macaque monkey were used in this work. The first experimental block included a Lithium-Pilocarpine model of epilepsy: LiCl (127 mg/kg) was injected (i.p.) to the animals the day before the actual experiment. The experiment started with anesthesia administration to the rats (5% sevoflurane for induction 1.5-2.5 for maintenance). Once a stable EEG recording was achieved Scopolamine (1mg/kg) was injected. Thirty minutes later, two doses of pilocarpine (20 mg/kg), 30 minutes apart, were administered. During each session two animals were recorded simultaneously and were classified as “magnetic” (a magnetic neodymium nickel-plated cylinder, magnetic field of 0.5 T, was placed over the skull before pilocarpine injection) or “sham” (a stainless steel replica without magnetic properties was used as the sham). In a second experimental block, we recorded spontaneous and visually induced epileptic-like activity (consequence from a cortical lesion after multiple microelectrode penetrations), in the visual cortex of a monkey (Macaca mulatta) in control conditions and during the presence of the magnet.

Results The main consequences of SMF application were a reduction and a delay in development of epileptic signs. Between 15-30 minutes after the second injection of pilocarpine, EEG changes compatible with epileptic seizures were clearly observable in the Sham animals: Electroencephalographic Down states almost disappeared and were substituted by an abnormal oscillatory activity with an increment in the EEG power at the 1-8 Hz band. Similar effects, although clearly attenuated, were visible in those animals under real stimulation but 1-2 hours later. In the monkey, we recorded spontaneous and visually-induced paroxysmal activity. After the application of SMF (30 minutes) over the cortical focus, abnormal activity was clearly reduced: the threshold for visual induction increased, and the severity and duration of the episodes were reduced.

Significance The results reinforce the view that SMF reduce the cortical excitability and suggest that SMF could be considered in the future as an adjuvant antiepileptic treatment.

Neuromodulation, epilepsy, magnetic fields

O4-02

BIOACTIVE LYSOPHOSPHOLIPIDS AND NEUROTRANSMITTERS REGULATE MOTONEURON INTRINSIC EXCITABILITY VIA RHO-KINASE

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Intrinsic membrane excitability (IME) influences neuronal plasticity and vulnerability to excitotoxic death. IME has a relevant impact in physiological processes such as, learning and adaptation to the changing external environment, and in pathogenic events linked to many neurological and psychiatric conditions. Therefore, identifying molecular mechanisms which control and/or determine neuronal IME is a matter that merits attention. In this way, Rho-associated kinase (ROCK) emerges as a firm candidate to effect on neuronal IME by its regulatory role on several ionic channels. In particular, ROCK phosphorylates and, subsequently, regulates the background K⁺ channel TASK1, which is highly expressed in

motoneurons (MNs). Interestingly, both, ROCK α and ROCK β isoforms, are expressed in the hypoglossal motor nucleus (HN), and ROCK α , especially, is highly expressed by MNs. However, results from our group showed that baseline ROCK activity, if any, did not impact on MNs IME *in vitro*. In this context, lysophosphatidic acid (LPA), a bioactive membrane-derived phospholipid, acting on G-protein coupled receptors (LPAR), downstream stimulates ROCK. Given that, as we recently reported, the isoreceptor LPA1 is highly expressed in MNs, we used here a saturated form of LPA (sLPA, 40 μ M) as a feasible option to stimulate ROCK in MNs. Accordingly, sLPA increased ROCK activity in the HN of rat pups. sLPA-triggered ROCK stimulation was prevented by co-addition of a specific ROCK inhibitor (H1152, 20 μ M). Whole cell patch-clamp recordings reported that sLPA induced membrane potential depolarization, reduced threshold current to evoke an action potential, and increased input resistance, i.e., increased IME, of MNs via ROCK α , but not via ROCK β . Furthermore, sLPA increased IME in wild-type MNs and in cells deficient in TASK3 (*task3*^{-/-}), another leak K⁺ channel highly expressed in MNs. Conclusively, sLPA-evoked changes on these parameters were fully absent in MNs lacking TASK1 (*task1*^{-/-}). Outstandingly, 5-HT and thyrotropin-releasing hormone (TRH), which regulate MNs IME by inhibiting TASK channels, also stimulated ROCK in the HN. In addition, H1152 almost fully avoided 5-HT- and TRH-induced inwardly-directed currents in MNs. Furthermore, addition to the recording solution pipette of active ROCK α mimicked sLPA effects on IME. Overall, these outcomes suggest that ROCK α regulates IME by inhibition of TASK1 channels in MNs. ROCK α is likely a partner in a common molecular mechanism which mediate the control of MNs IME by lysophospholipids and neurotransmitters that target G-protein coupled receptors.

Motoneurons, excitability, neurotransmitters, Rho-kinase, TASK channels

O4-03

MORPHOFUNCTIONAL STUDY OF TRANSMITTER RELEASE USING HIGH PRESSURE FREEZING AND ELECTRICAL STIMULATION IN CULTURED RAT HIPPOCAMPAL NEURONS

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Neurotransmission is the key process in neuronal communication triggered by calcium influx at the presynaptic terminal upon the arrival of an action potential. Calcium entry binds to proteins that cause the fusion of synaptic vesicles with the presynaptic membrane and transmitter release. The molecular machinery driving vesicle fusion is well known, however how and where synaptic vesicles fuse at a particular synapse is still a matter of debate. Also, in many central neurons the probability of vesicle fusion is between 0,1 and 0,3, indicating that an action potential does not always cause the fusion and release of a single packet of neurotransmitter to the synaptic cleft. We aim to correlate physiological data of vesicle fusion probability with electron microscopy. We have used a technique that couples single electrical field stimulation with high-pressure freezing, ‘zap-and-freeze’. We used zap-and-freeze to visualize vesicle fusion and the reorganization of synaptic vesicle pools with millisecond resolution at cultured mouse hippocampal synapses by electron microscopy. Our results indicate that upon stimulation of a single stimulus (1 AP) the number of vesicles in the presynaptic density is similar, or slightly increases with respect to the control, unstimulated situation. The application of 40 AP stimulation (to deplete the Readily Releasable Pool) caused a decrease in the number of tethered vesicles (between 50 and 100 nm from the

presynaptic density) to the membrane, with no change in the number of docked vesicles (less than 10 nm). Only with high intensity stimulation (600 AP) we observed a depletion of docked and tethered vesicles. We rarely observed Ω shapes after the application of single stimulation. Taken together, our results suggest that the presynaptic density and vesicles adjacent to it keeps a very well define structure with single or moderate stimulus, being altered only with a continuous burst of AP.

Synapse, exocytosis, high-pressure-freezing, electron-microscopy

O4-04

COMPLEX INTERACTIONS OF TETRACAINE WITH MUSCLE-TYPE NICOTINIC RECEPTORS

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The main targets of local anesthetics (LAs) are voltage-dependent Na⁺ channels, thus preventing the action potential generation in nerve cells. Besides, most LAs modulate the activity of other ion channels, including ligand-gated ion channels (LGIC), as the nicotinic acetylcholine (ACh) receptor (nAChR). Therefore, it is of therapeutic relevance to understand the mechanisms by which LAs modulate nAChRs and other LGIC, since these interactions might account for some of the side effects found when LAs are used in the clinical practice. We previously showed that lidocaine acts on nAChRs by different means (Alberola-Die et al. 2011; J. Neurochem. 117, DOI: 10.1111/j.1471-4159.2011.07271.x), which we later related to its presence as charged and uncharged forms in physiological solutions (Alberola-Die et al. 2016 a,b; Front. Mol. Neurosci. 9, DOIs: 10.3389/fnmol.2016.00012 and 10.3389/fnmol.2016.00127). We have now studied the mechanisms of interaction of the LA tetracaine (Ttc), which at physiological pH is almost exclusively present as a positively charged molecule, on nAChRs. Torpedo nAChRs were transplanted to *Xenopus* oocytes and currents elicited by ACh (IACHs), either alone or co-applied with Ttc were recorded. In addition, *in silico* docking assays of nAChR-Ttc interactions were performed. We have found that Ttc binds to nAChRs at multiple sites, involving extracellular and transmembrane domains, in both open- and closed-nAChR conformations. Our functional results show that Ttc is a powerful blocker of muscle-type nAChRs, showing an IC₅₀ in the submicromolar range. At low Ttc concentrations, below the IC₅₀, IACH inhibition was only present at negative potentials, indicating open-channel blockade. At higher Ttc concentrations, IACH decay was hastened, suggesting an enhanced desensitization. Interestingly, the acceleration of desensitization could be prevented by holding the cell at positive potentials, which should eject Ttc from inside the pore. Besides, Ttc concentrations above the IC₅₀ elicited IACH inhibition both at negative and at positive potentials, suggesting the presence of an additional closed- (resting) channel blockade. Taking into account the virtual docking findings, our functional results suggest that extracellular binding sites are relevant for the closed-channel blockade whereas two sites located within the channel pore, showing different affinities for Ttc, would contribute to open-channel blockade and enhancement of nAChR desensitization, respectively. It is worth noting that Ttc modulates nAChR function at concentration far below those required to block voltage-dependent Na⁺ channels. Funding by: BFU2012-31359, SAF2015-66275-C2-1-R and SAF2017-82977-689 P (AEI/FEDER, UE) from MINECO and PROMETEO/2014/11 from GV (Spain). R.C. held a predoctoral fellowship from UA (FPUUA36).

Tetracaine, nAChRs, modulation, virtual docking

O4-05

NON-TUMOROGENIC DITERPENES PROMOTE NEURAL STEM CELLS PROLIFERATION AND NEUROGENESIS

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Aims: Several neurological disorders are associated with an irreversible loss of neurons. There is no effective treatment nowadays to facilitate replacement of the lost neurons, however, strategies based on the promotion of neurogenesis from endogenous neural stem cells are potential therapeutic options to treat this type of disorders. New neurons, in the adult brain are generated under physiological conditions throughout the entire life, controlled by extracellular signalling molecules coupled to intracellular cascades. Proteins participating in these cascades constitute potential pharmacological targets to promote neuronal replacement. Among these proteins the kinases of the protein kinase C (PKC) family constitute promising targets. The general activation of PKC with PMA promotes neural progenitor cells (NPC) proliferation. In addition, other non-tumour promoting diterpenes with phorboid structure such as prostratin or other 12-deoxiphorbols facilitate NPC proliferation *in vitro* and *in vivo* via PKC activation, suggesting a role for these molecules as therapeutic drugs to promote neuroregeneration. Unfortunately, bioavailability of these diterpenes is limited in nature and finding new sources of these compounds would be of great interest. In order to find a solution to this problem, our group has isolated from croton oil molecules with modifiable structures, which could mimic the effect of prostratin. Our study analyzes the effect of these semi-synthetic compounds in modulating NPC proliferation and neurogenesis.

Methods: We have tested three diterpenes with a prostratin skeleton. *In vitro* analysis of the effect of these compounds on proliferation and differentiation were performed using neurospheres obtained from post-natal mice (P7). *In vivo* analysis of the effect on neurogenesis were performed by administering intranasal doses of the compounds for 3 and 7 days and studying neurogenesis afterwards. Care and handling of animals were performed according to the Guidelines of the European Union Council (2010/63/EU), and following the Spanish regulations (65/2012 and RD53/2013) for the use of laboratory animals.

Results: Diterpenes tested promoted NPC proliferation both *in vitro* and *in vivo* and could have a protective effect on NPC cultures. No effect on differentiation was observed with any of the molecules tested in this study.

Conclusion: Our results support the potential of molecules with phorboid structure as new pharmaceutical agents to facilitate neuronal renewal.

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Neurogenesis, PKCs, diterpenes, neural progenitor cell

O4-06

IMPLICATION OF THE NORADRENERGIC SYSTEM (LC-BLA PATHWAY) IN AFFECTIVE DISORDERS AND COGNITION IN A MODEL OF CHRONIC PAIN IN RATS USING DREADDS

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Chronic pain affects more than 20% of the population in Europe and 20-30% of them suffer disorders related to anxiety, as well as cognitive deficit reaching a greater magnitude the problem. However, the neurobiological mechanisms involved in this comorbidity are unknown. Preclinical studies suggest that the locus coeruleus (LC), the main noradrenergic nucleus, has a relevant role in the development of these disorders associated with chronic pain. One of its main projections is directed towards the basolateral amygdala (BLA), a key nucleus in the formation of aversive memory and in the development of affective disorders associated with chronic pain. Thus, in this study we propose that chronic pain produces an activation of the LC-BLA pathway that promotes the appearance of affective disorders and cognitive alterations. To address this hypothesis, we have evaluated the implication of the activation or inhibition of the LC-BLA pathway using a DREADDs pharmacogenetic technique (Designer Receptor Exclusively Activated by Designer Drugs) on the development of anxiety and cognitive alterations in an animal model of long-term neuropathic pain. Here, we characterized the sensory, emotional and cognitive consequences of neuropathic pain in a rat model. The results of the study have shown that the pharmacogenetic inhibition of the LC-BLA pathway reverses anxiety and blocks the improvement of aversive learning in long term neuropathic pain animals. Interestingly, the activation of the LC-BLA pathway induces anxiety and an increase in aversive learning in sham animals while maintaining the anxiety-like behavior and aversive learning manifested by animals subjected to long-term neuropathic pain. However, no sensory or cognitive changes were observed in relation to attention and in visuospatial recognition depending on the inhibition or activation of the LC-BLA pathway. With this study we conclude that long-term neuropathic pain produces a hyperactivation of the LC projections towards the BLA, promoting the appearance of anxious behavior and cognitive alterations. It should be noted a greater processing of aversive stimuli, which can contribute to a greater vulnerability to stress, being critical for the development of post-traumatic, affective or anxiety disorders. Therefore, the study of specific pathways involved in chronic pain brings us closer to the development of new therapeutic targets.

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Oral session 5: Endocrinology, Metabolism & Nutrition

O5-01

CDK2: A NEW PLAYER IN ADIPOSE TISSUE METABOLISM?

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Obesity is a worldwide health problem affecting millions of people, characterized by an excessive fat accumulation, which increases the risk of suffering several metabolic diseases including type 2 diabetes. Lately, cyclin dependent kinases (CDKs) have emerged as possible actors in obesity development. CDKs not only take part in cell cycle regulation, but also control specific metabolic processes like insulin secretion (pancreas), gluconeogenesis (liver) or insulin resistance (adipose tissue). Specifically, the aim of this work was to understand whether CDK2 plays a function in the metabolism of mature adipocytes and obesity. Microarray analysis of our group demonstrated that CDK2 was expressed in human subcutaneous adipose tissue (SAT). Moreover, CDK2 protein was detected in mature isolated adipocytes (a typical example of non-proliferative cell type), suggesting a potential function in adipocyte metabolism. Besides, studies in mice indicated that CDK2 protein levels were increased in SAT of obese mice. Furthermore, studies in 3T3-L1 adipocytes treated with a CDK2 inhibitor, showed impaired insulin sensitivity, affecting main cellular processes in adipocytes such as glucose uptake, glycerol release and lipid content. Together, these results pointed CDK2 as a major regulator of adipose metabolism. However, further investigations are necessary to decipher the specific molecular mechanisms controlled by CDK2.

Cdk2, metabolism, adipocytes

O5-02

TARGETING BROWN ADIPOSE TISSUE IN OBESITY AND DIABETES

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Obesity is growing at an alarming rate and in parallel to the increase of its associated metabolic diseases such as type 2 diabetes, insulin resistance, cardiovascular disease and some forms of cancer. The discovery of active brown adipose tissue (BAT) in adult humans and that it is reduced during obesity and diabetes has put a spotlight on this tissue as a therapeutic target in obesity-induced metabolic disorders. As opposed to the energy-storing white adipose tissue (WAT), BAT utilizes fuels such as fatty acids to produce heat and control body temperature in a process called thermogenesis. The mechanisms involved in the development of obesity-induced metabolic diseases have been associated to WAT dysfunction such as fibrosis, apoptosis, inflammation, ER and oxidative stress. However, little is known of whether these processes are also present in BAT during obesity. Our aim was to characterize the BAT of obese and hyperglycemic mice treated with a high-fat diet (HFD) for 20 weeks. The hypertrophic BAT from obese mice showed elevated levels of inflammation, ER stress, ROS generation and antioxidant enzyme activity compared to lean controls. The response was attenuated compared with obesity-induced WAT derangements, which suggests that BAT is more resistant to the obesity-induced insult. In fact, mitochondrial respiration measured with the

Seahorse analyzer in BAT from obese mice was enhanced, with a 2-fold increase in basal oxygen consumption, through the upregulation of complex III of the electron transport chain and UCP1. In conclusion, our results show that obesity is accompanied by an increase in BAT mitochondrial activity, inflammation and oxidative damage.

Obesity, brown adipose tissue, inflammation

O5-03

HYPOTHALAMIC ER STRESS AS NEW TARGET AGAINST OBESITY AND METABOLIC ALTERATIONS

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Introduction: Obesity and its associated pathologies, such as diabetes, cardiovascular disease, or some cancer types, have an increasing prevalence, being considered a disease by the HWO. Obesity has been related with alterations in endoplasmic reticulum (ER) alterations in peripheral tissues such as liver, pancreas or skeletal muscle (Ozcan et al., 2009; Hosoi et al., 2008; Ropelle et al., 2010; Martínez de Morentin and López, 2010; Cnop et al., 2012), and more recently to central level (Schneeberger et al. 2013, Contreras et al. 2014). The chaperone GRP78/Bip (glucose regulated protein 78 kDa/binding immunoglobulin protein) acts upstream the unfolded protein response (UPR) to modulate protein folding in reply to cellular insults that lead to ER stress.

Aim: The aim of this study was to investigate the role of hypothalamic ER stress in the control of energy balance during several obesity degrees, specially the involvement in the activation of the thermogenesis in the brown adipose tissue (BAT) and in the white adipose tissue (WAT). **Methods:** Different obesity models were stereotaxically treated with adenoviruses encoding GRP78/BiP specifically in the ventromedial nucleus of hypothalamus (VMH), improving the protein folding so reducing the ER stress. Metabolic parameters and thermogenesis in BAT and WAT were determined.

Results: Here, we demonstrate that reduction of hypothalamic ER stress induces body weight loss and improvement in metabolic parameters, independent on food intake, through increased thermogenesis in BAT and WAT. This effect was greater in stronger obesity models while there was not effect in control rats.

Conclusion: This evidence indicates that modulation of GRP78 activity (and then reducing ER stress) in specific hypothalamic nuclei may be a potential strategy against obesity and associated comorbidities.

ER stress, obesity, hypothalamus

O5-04

OVERFEEDING DURING LACTATION IN SPRAGUE-DAWLEY RATS INDUCES CARDIOVASCULAR INSULIN RESISTANCE IN ADULTHOOD

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Nutrition during early life plays a key role in the development of metabolic and cardiovascular alterations in adult life. These alterations may be the result of decreased insulin sensitivity not only in metabolic tissues but also in the cardiovascular system, where insulin exerts direct effects inducing arterial vasodilation and positive inotropic effects in the myocardium. This work aims to evaluate the effects of early overnutrition in the development of cardiovascular insulin resistance in the long term in male Sprague-Dawley rats. For that purpose we used an experimental model of childhood obesity in rats. At birth rats were organized either in litters of 12 pups/mother (C12-Controls) or in 3 pups/mother (C3-Overfed) with no other manipulation to offsprings during the 21-day-lactation period. After weaning, rats were housed 3 per cage and fed ad libitum until sacrifice at the age of 6 months. After sacrifice, hearts were set into a Langendorff system whereby increasing insulin doses were administered and coronary perfusion pressure, heart rate and heart contractility were recorded. Likewise, the aorta was dissected, cut in 2mm segments and set in an organ bath whereby changes in vascular tension in response to increasing insulin concentrations were recorded. Finally to assess the activation of the two main pathways involved in insulin intracellular signalling, total proteins were obtained from myocardial and arterial tissues and the MAPK and Akt expression and activation in response to insulin were analysed. In the vascular reactivity experiments insulin induced a higher vasodilation in aorta segments from C3 rats compared to C12. However, this fact was not associated with the molecular analysis as the p-Akt/Akt ratio was diminished in the arterial tissue of C3 compared to C12 rats and the p-MAPK/MAPK ratio showed no changes. In the heart, no significant changes were found in coronary perfusion and intraventricular pressures in response to insulin administration. However insulin significantly increased heart rate at the dose of 10-7 M in C3 compared to C12 and induced a lower increase in heart contractility in hearts from overfed rats compared to controls at the dosages of 10-9M and 10-8M. These changes were associated with decreased p-Akt/Akt and increased p-MAPK/MAPK ratios in response to insulin in the myocardium of overfed rats. In conclusion, overfeeding during lactation in rats induces cardiovascular insulin resistance in adulthood with this fact possibly being related with the reported cardiovascular alterations reported in this experimental model.

Childhood obesity, cardiovascular, insulin resistance

O5-05

ALTERED HOMOCYSTEINE METABOLISM IN A MIXED MODEL OF ALZHEIMER'S DISEASE AND METABOLIC DISEASE

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Hyperhomocysteinemia (HHcy), abnormally elevated levels of plasma homocysteine (Hcy), has been linked to damage on the central nervous system in different neurodegenerative disorders. Specifically, plasma levels of Hcy are elevated in patients with Alzheimer's disease (AD). Moreover, HHcy has also been linked to disorders such as cardiovascular disease, obesity or type 2 diabetes. Nevertheless, the underlying mechanisms for this complex relationship including AD-metabolic disease and HHcy have not been elucidated. Hcy is produced in all living cells as a byproduct of methionine metabolism. Dietary methionine is metabolized in the liver producing the cellular methyl donor S-adenosylmethionine

(SAME). The use of SAME in methylation reactions leads to the indirect production of Hcy, which can be eliminated through the transsulfuration pathway or remethylated to methionine, producing the amounts of SAME required to perform all cellular methylation reactions. HHcy has also been linked to the development of other disorders such as cardiovascular disease, obesity, and type 2 diabetes, also related to AD. Although age remains the main risk factor for developing AD certain metabolic alterations, including prediabetes and hyperinsulinemia, also increase this risk. All these findings highlight the existence of a close relationship between AD, liver methionine metabolism and hyperinsulinemia. We have studied in here liver methionine metabolism in a mouse model of AD (APP^{swe}/PS1^{dE9} mouse) with severe hyperinsulinemia induced by high fat diet (HFD) treatment. Prolonged HFD induces a pre-diabetic condition, which facilitates the study of the interaction AD-prediabetes. AD pathology and HFD provoke alterations in liver methionine metabolism, which more severe in AD mice fed with HFD for a prolonged period of time. The expression of methionine adenosyltransferase is upregulated in AD mice and this increase is enhanced by HFD. Upregulation in expression of DNA methyltransferases, enzymes participating in Hcy remethylation and transsulfuration, as well as amyloid β peptide-degrading enzyme (IDE) can be found in AD mice fed with HFD. Liver histopathology of AD and HFD mice is compatible with moderate steatosis, whereas in AD mice fed with HFD histology is compatible with fatty liver pathology. Peroxisome proliferator-activated receptor (PPAR)-alpha, a ligand-activated transcriptional factor, is upregulated in AD mice fed with HFD. PPAR-alpha regulates the expression of genes involved in fatty acid beta-oxidation and is a major regulator of energy homeostasis. All these results suggest that prediabetes induces compensatory alterations in liver methionine metabolism in an attempt to maintain plasma Hcy levels within a normal range avoiding fatty liver pathology.

Alzheimer's disease, diabetes, homocysteine

O5-06

DYNAMICS OF THE PANCREATIC ALPHA-CELL MASS AT THE RECENT ONSET OF EXPERIMENTAL AUTOIMMUNE DIABETES

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Introduction: There is a growing interest in the study of the pancreatic alpha-cell and its role in the pathogenesis of autoimmune diabetes. Glucagon secretion is impaired in type 1 diabetic patients. Additionally, emerging evidence indicates that the alpha-cell can be a source to repopulate the ablated beta cell mass in diabetes. To start clarifying the alpha-cell adaptations to autoimmune diabetes, we investigated the main indicators of its mass balance at the recent onset of experimental autoimmune diabetes (EAD) in the RIP-B7.1 mouse model background. **Methodology:** Pancreata from RIP-B7.1 immunized mice and age-sex matched littermate controls were removed one week after the diabetes onset to perform immunohistochemical studies.

Results: At this stage of EAD, diabetic mice show hyperglucagonemia and a high lymphocytic infiltration within the islets along with a great reduction of insulin blood levels and total insulin pancreatic content. In this inflammatory environment, the alpha-cell shows hypertrophy and its mass is preserved. Moreover, diabetic mice do not present significant changes in apoptotic alpha-cell percentage while the alpha-cell

neogenesis and proliferation rate were increased.

Conclusions: The alpha-cell capacity of survival and self-renewal appears to be increased in autoimmune diabetes, opening a new focus to understand its implications in the pathology.

Alpha-cell, Autoimmune diabetes, RIP-B7.1 mice

Oral session 6: Cardiovascular II

O6-01

WHY HIGH LEVELS OF ENDOGLIN ARE ASSOCIATED WITH A WORSE PROGNOSIS IN PATIENTS WITH CANCER?

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Angiogenesis is the de novo formation of blood vessels from preexisting ones. This process occurs physiologically, but it may also be essential in some diseases, such as cancer. Since it has been shown that blood vessel formation is essential for tumor growth, quantification of microvasculature density is a common practice for predicting tumor prognosis. More specifically, a high number of endoglin-positive vessels is associated with a poor prognosis in several solid tumors. Endoglin is a membrane glycoprotein whose absence or deficiency produces alterations in angiogenesis and, therefore, a lower tumor growth. For this reason, it is widely accepted that high levels of endoglin produce a worse prognosis due to larger tumors. The aim of this work was to analyze the effect of endoglin overexpression on angiogenesis and tumor growth. For this purpose, we used mice that overexpress human endoglin (ENG+). After subcutaneous injection of Lewis lung carcinoma (LLC) cells in these mice, we observed that tumors developed in these mice are not larger nor have a greater number of vessels than those of WT mice. However, we observed large regions of erythrocytes infiltrated between the tumor cells in ENG+ mice that are not present or they are very small in WT mice, suggesting alterations in angiogenesis. These alterations in angiogenesis were confirmed by analyzing the development of the retinal vasculature in these mice and by Direct In Vivo Angiogenesis Assay (DIVAA). In addition, we have analyzed, both in vivo and in vitro, several phases of angiogenesis and conclude that continuous endoglin overexpression mostly affects maturation and stabilization of the new vessels, that can result in leaky vessels. In the case of tumors this has a special interest since it facilitates the tumor cell intravasation and the generation of metastasis, as we demonstrate in our model. In summary, in this work we show that worse prognosis in tumors associated with the high levels of endoglin is not due to greater angiogenesis that increase tumors growth, but it produces defective vessels that facilitate the passage of tumor cells to the blood and the generation of metastasis, which are the primary cause of the worse prognosis.

Endoglin, angiogenesis, cancer, metastasis

O6-02

AMPK INDUCES RENAL VASODILATION AND REDUCES OXIDATIVE STRESS IN OBESITY

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The adenosine monophosphate-activated protein kinase (AMPK) is a key enzyme that acts as a cellular energy sensor. AMPK is activated by different stress signals such as exercise, fuel deprivation, hypoxia or drugs and stimulates catabolic pathways to produce energy and restore homeostasis. In addition to its function on metabolism, vascular AMPK has recently been identified as a potent vasodilator in resistance arteries (Schneider et al., *Hypertension* 2015; 66:108). Obesity leads to renal endothelial damage and AMPK dysregulation and is a major risk factor for the development of chronic kidney disease (García-Prieto et al., *Vascular Pharmacol* 2015; 67-69). Activation of AMPK might be an important target to improve vascular function in these diseases. In the present study we sought to assess the role of vascular AMPK in renal resistance arteries of a rat model of genetic obesity. Renal interlobar arteries isolated from the kidney of male obese Zucker rats (OZR) and their control counterparts, lean Zucker rats (LZR) were mounted in microvascular myographs. Concentration-responses curves to acetylcholine (ACh) and isoprenaline were performed to assess function. Arterial segments with intact endothelium or endothelium-denuded pre-contracted with phenylephrine (Phe) were treated with the AMPK selective activator (A769662). In some experiments, the PI3K inhibitor (LY-294002) or the NOS inhibitor (L-NOARG) were added 25 min before pre-contraction with Phe. Changes in basal and NADPH-stimulated levels of superoxide were measured by lucigenin chemiluminescence in renal arteries and in renal cortex of LZR and OZR. Endothelium-dependent relaxations to ACh and isoprenaline were reduced in OZR compared to LZR. The AMPK activator A769662 elicited relaxations of lesser magnitude in OZR compared to LZR, although treatment with low concentrations of the A769662 improved ACh-mediated vasodilation and endothelial function in OZR. Mechanical endothelium removal and the inhibition of PI3K or eNOS markedly reduced the relaxations induced by AMPK activator in LZR but not in OZR. Regarding oxidative stress, basal superoxide levels were markedly enhanced by NADPH addition in arteries and cortex of LZR and OZR. NADPH-stimulated superoxide levels were higher in OZR compared to LZR and blunted by the AMPK activator in renal interlobar arteries and renal cortex in both strains. These results suggest that endothelium-dependent vasodilator mechanism of AMPK involve the AMPK-PI3K-eNOS pathway, and this pathway is impaired in obesity, although AMPK actions in vascular smooth muscle are preserved. Moreover, treatment with AMPK activator reduces oxidative stress in arteries and renal cortex in obesity and improves endothelial function.

Endothelial dysfunction, oxidative stress, obesity, AMPK

O6-03

VASCULAR REMODELLING AND STIFFENING INDUCED BY FETAL PROGRAMMING (SPRAGUE DAWLEY RATS)

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Background. Suboptimal fetal growth, and subsequent low birth weight, are associated with a higher incidence of hypertension. In the rat, maternal nutrient restriction during gestation induces hypertension only in male

offspring⁽¹⁾. One of the proposed mechanisms is a defective vascular development, and, in particular elastogenesis, which might compromise the vascular mechanical properties from early life⁽²⁾.

Hypothesis and aims. Early vascular remodeling and impaired elastogenesis may explain hypertension development in adult males exposed to fetal undernutrition. We aimed to assess the role of aortic structural and mechanical alterations in a rat model of hypertension programming, evaluating changes at birth, weaning and adult life.

Methods. Rats were fed ad-libitum (control) or with 50% of the control daily intake during the second half of gestation (maternal undernutrition, MUN). 3-day, 21-day and 6-month old male and female offspring was studied. Blood pressure was evaluated in vivo. In thoracic aorta we assessed gross structure (confocal microscopy), mechanical properties of whole segments and purified elastin (pressure myography and isometric tension recording), collagen and elastin content (hot alkali elastin purification and weight and dot blot-based Sirius red collagen quantification), and internal elastic lamina (IEL) organization (confocal microscopy).

Results. Only adult MUN male offspring developed hypertension. At birth MUN rats were lighter and their aorta was smaller; during lactation MUN offspring exhibited catch-up growth and aorta hypertrophy similar in males and females, which was maintained in adult rats. MUN aorta was more compliant in 3-day and 21-day old rats associated with lower collagen content and larger fenestrae in the IEL, returning to normal in adulthood. Purified elastin from young MUN offspring was more compliant in both sexes; only MUN adult females maintained larger elastin compliance.

Conclusions. Fetal undernutrition is associated with deficient aortic development followed by hypertrophic remodeling due to accelerated perinatal growth, and larger aortic compliance, likely related to alterations in collagen and elastin deposition. These modifications argue against a mechanical cause for the sex differences in hypertension development associated with fetal growth restriction induced by maternal undernutrition⁽³⁾.

Acknowledgements. This work was supported by MINECO/FEDER (FEM2015-63631-R).

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Elastin, collagen, vascular mechanics

O6-04

PARTICIPATION OF THE LATE SODIUM CURRENT IN THE INDUCIBILITY OF VENTRICULAR FIBRILLATION DURING VENTRICULAR LOCAL STRETCH

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Introduction: Acute ventricular stretch is arrhythmogenic and the participation of late sodium current (INaL) in the intrinsic electrophysiological effects due to stretching and its relationship to the establishment of life-threatening arrhythmias such as ventricular fibrillation (VF) is not well known.

Purpose: The aim of the present work is to investigate, in an isolated rabbit heart, the possible involvement of INaL in the arrhythmogenic

effect of stretching through the analysis of the inducibility of ventricular fibrillation, and to determine the possible participation of modifications of the refractoriness, by INaL blocking with eleclazine.

Methods: 13 New Zealand male rabbits were heparinized and sacrificed with sodium thiopental. The hearts were excised and located in a Langendorff system to perfusion and metabolic support. A stimulation electrode and 110 monopolar recording electrodes were placed on the ventricular epicardium. The stimulation was carried out by means of a Grass S88 stimulator and the epicardial recordings were obtained using a cardiac electrical activity mapping system (MapTech). To determine, during stretch, the inducibility of the VF, the effective refractory period (ERP) and the length of the cycle previous to that triggered the VF (LCPVF), as an approach measure of ERP, the ventricular extrastimulus test was applied. Acute local stretch in the left ventricle was produced by introducing an ad hoc device. We compared the inducibility of VF (during ventricular stretch), prior to drug administration, in control situation (C) and after cessation of drug administration and its elimination by flushing (F), compared to the situation: treatment with eleclazine (GS6615); that is, C + F versus GS6615. To know the effect of stretching on refractoriness, the LCPVF was compared with ERP in C + F situation with the ERP after GS6615 treatment.

Results: 6 C + F of 13 hearts, fibrillated, and none of the GS6615 hearts. Regarding the refractoriness during ventricular stretching, we found that, in ms, LCPVF of C + F was significantly lower ($p < 0.05$) than the ERP of GS6615 (95 ± 15 , $n = 6$, vs 118 ± 14 ; $n = 5$).

Conclusions: INaL current seems to be involved in the arrhythmogenic effect of the ventricular acute local stretch, being able to operate, at least in part, avoiding the shortening of refractoriness by stretching.

Acknowledgements: This work was supported by PI15/01408 and CIBERCV CB16/11/0486 ISCIII and FEDER funds.

Myocardial stretch, late sodium current, arrhythmias

O6-05

DOES DRONEDARONE IMPROVE STRUCTURE AND FUNCTION OF CORONARY ARTERIES? EXPERIMENTAL STUDY IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objectives: Arterial hypertension is one of the most important causes of atrial fibrillation (AF) occurrence. Dronedarone is a multichannel blocker used for the treatment of AF. However, its effect on arterial remodelling has not yet been demonstrated. The present study aims to analyze the effect of dronedarone in coronary vascular remodeling.

Materials and Methods: we treated adult male spontaneously hypertensive (SHR) rats with dronedarone (SHR-D, $n=9$) or placebo (SHR, $n=9$). As normotensive controls we used Kyoto rats (WKY, $n=9$). After 14 days of treatment, left anterior descending coronary arteries were dissected. Segments of each artery were mounted on a wire myograph. To study vasoconstrictor responses, concentration-response curves to 5-hydroxytryptamine (5-HT) were performed (3×10^{-8} to 3×10^{-5} mol/L). We evaluated vasodilator responses with increasing concentrations of acetylcholine (ACh 10^{-9} to 10^{-4} mol/L) in segments precontracted with serotonin (5-HT 3×10^{-7} mol/L). Vascular structure was analyzed by confocal microscopy (wall thickness, media and adventitial layers).

Comparisons among groups were made by ANOVA test. All data was expressed as mean \pm SEM. $P < 0.05$ was considered significant. All procedures were approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Results: Dronedaronone improved vasodilator responses at low doses of acetylcholine (10^{-9} to 10^{-7} mol/L) and decreased vasoconstrictor responses compared to SHR (10^{-7} to 3×10^{-5} mol/L). Wall ($p < 0.001$), adventitial ($p < 0.001$) and media thickness ($p < 0.05$) were decreased in SHR-D compared to SHR. Cell number was decreased in SHR-D in both adventitial ($p < 0.05$) and media layer ($p < 0.01$) compared to SHR. No statistical differences were observed between WKY and SHR-D either in function or structure.

Conclusions: we observed an early regression of coronary arteries remodelling after treatment with dronedaronone in an experimental model of arterial hypertension.

Acknowledgements: This work was supported by a grant from FIS 13/01261 and Fondos FEDER, Spain.

Dronedaronone, arterial hypertension, coronary arteries

O6-06

NADPH OXIDASE 4- AND 2-DERIVED HYDROGEN PEROXIDE IS INVOLVED IN THE ENDOTHELIUM-DEPENDENT VASODILATATION OF INTRARENAL ARTERIES

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The role of NADPH oxidase (Nox)-derived reactive oxygen species in kidney vascular function has extensively been investigated in the harmful context of oxidative stress in diabetes and obesity-associated kidney disease (Ratliff et al., *Antioxid Redox Signal* 25:119-146, 2016 and Sharma, *Antioxid Redox Signal* 225: 208-216, 2016). Since hydrogen peroxide (H_2O_2) has recently been involved in the non-nitric oxide non-prostanoid relaxations of intrarenal arteries (Muñoz et al., *Free Radic Biol Med* 106:168-183, 2017), the present study was sought to investigate whether NADPH oxidases may be functional sources of vasodilator H_2O_2 in the kidney and to assess their role in the endothelium-dependent relaxations of human and rat intrarenal arteries. Renal interlobar arteries isolated from the kidney of renal tumor patients who underwent nephrectomy, and from the kidney of Wistar rats, were mounted in microvascular myographs to assess function. Superoxide and H_2O_2 production was measured by chemiluminescence and Amplex Red fluorescence, and Nox2 and Nox4 enzymes were detected by Western blotting and by double immunolabeling along with eNOS. Under conditions of cyclooxygenase and NO synthase blockade, acetylcholine induced catalase-sensitive endothelium-dependent relaxations that were blunted by the non-selective Nox inhibitor apocynin and by the Nox2 or the Nox1/4 inhibitors gp91ds-tat and GKT13690, respectively. Nox2 and Nox4 proteins were expressed in the endothelium of renal arterioles and glomeruli co-localized with eNOS, levels of expression of both enzymes being higher in the cortex than in isolated arteries. Acetylcholine stimulated H_2O_2 production that was reduced by gp91ds-tat and by GKT136901. These results suggest the specific

involvement of Nox4 and Nox2 subunits as physiologically relevant endothelial sources of H_2O_2 generation that contribute to the endothelium-dependent vasodilatation of renal arteries and therefore have a protective role in kidney vasculature.

Endothelium-mediated vasodilatation, Nox4, hydrogen peroxide

Oral session 7: End-of-Degree Projects

O7-01

IMPLEMENTATION OF A DESIGN FOR AN ELECTROPHYSIOLOGICAL STUDY OF SUPRA-ENCEPHALIC MECHANISMS INVOLVED IN THE CONTROL OF LARYNGEAL ACTIVITY AND SUBGLOTTIC PRESSURE

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Background: abduction and adduction of the vocal folds are performed by two populations of motoneurons located in the caudal portion of the nucleus Ambiguus (nA). In the rat, nA can be divided into three parts: compact formation (motoneurons innervating the esophagus), semicompact formation (motoneurons innervating the pharynx and cricothyroid muscle through the superior laryngeal nerve) and loose formation (motoneurons innervating the rest of laryngeal muscles). It is necessary to characterize the electrophysiological relationships between hypothalamic-mesencephalic-pontine neuronal circuits to understand their role in laryngeal pathophysiology and its effect on vocalization. **Objectives:** to characterize the role of the Dorsomedial Hypothalamic nucleus (DMH), the mesencephalic Periaqueductal Gray matter (PAG) and the pontine Parabrachial (PB) and A5 Region in the central mechanisms controlling laryngeal motoneuron activity and its role in vocalization. To achieve this objective is necessary to develop a variation of the classical technique of the “isolated glottis in situ” for the recording of subglottic pressure in rats.

Methods: Experimental basic preclinical study in non-inbred male rats. SPF, Sprague-Dawley (250-300 grams) housed under standard conditions have been used. Animals were anesthetized with sodium pentobarbital. A double tracheal (upwards in direction of the glottis for the “glottis isolated in situ” technique, and downwards in the direction of the carina) and esophageal cannulation were performed. Vagus and laryngeal recurrent nerves were isolated and stimulated with bipolar electrodes (Ag/AgCl). Bilateral parietostomy allowed access to the Hypothalamic Defense Area (HDA), PAG and nA. Electrical stimulation of the PAG and HDA using concentric bipolar electrodes was performed. The hypothalamic-mesencephalic-pontine neural circuits involved in the modulation of subglottic pressure and laryngeal activity were studied using extracellular neuronal recordings together with ortho and antidromic stimulation of these regions. Subglottic pressure, respiratory flow, pleural pressure, blood pressure, heart rate and unitary neuronal activity were also recorded. **Results:** Subglottic pressure was recorded in rats with an aneroid transducer (Hugo Sachs Elektronik D-7801, ± 0.1 psi) by passing a stream of humidified medical air upwards through the larynx at a constant rate of 50-100 ml/min with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V)

Conclusions: Our variation of the classical technique for the recording of the “isolated glottis in situ” in rats shows good dynamic

responses and can be perfectly used as an index of subglottic pressure and laryngeal activity.

Subglottic Pressure, Laryngeal Motoneurons, Nucleus Ambiguus

O7-02

EFFECT OF MITHRAMYCIN-A ON PRIMARY CULTURES OF SPINAL CORD MOTONEURONS UNDER EXCITOTOXIC STRESS. APPLICATION TO A MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower motor neurons (MNs), which results in skeletal muscle paralysis, and finally, death, usually within 2 to 3 years after the onset of symptoms. Glutamate-induced (Glu) excitotoxicity, which involves a deregulation of intracellular Ca^{2+} homeostasis, is one of the main mechanisms that contributes to MN degeneration in ALS. Remarkably, neurons are not equally vulnerable to excitotoxic damage, it depends on the physiopathological state in which they are. Thus, membrane hyper-excitability increases neuronal vulnerability to excitotoxicity. In our lab we unveiled a novel molecular pathway that exacerbates MN excitability and vulnerability in an ALS model. In summary, over-expression of the ubiquitous transcription factor Sp1 (specificity protein 1) increases p11 expression, which hampers plasma membrane expression of the leak K^+ channel TASK1, and, subsequently, increases MN excitability and vulnerability to an excitotoxic stimulus. Since mithramycin-A (Mit-A), an FDA-approved anti-cancer agent, inhibits Sp1 binding to DNA, we hypothesized on a feasible neuroprotective effect of Mit-A on MNs exposed to an excitotoxic stimulus. In this line, we analyzed Mit-A (30 nM) effects on both, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) dynamics by Ca^{2+} imaging, and the survival of primary cultures of spinal cord MNs (SMNs), isolated from mouse embryos, exposed to the excitotoxic agent Glu (150 μ M; 30 min). Outstandingly, Mit-A strongly attenuated Glu-induced disruption of intracellular Ca^{2+} homeostasis and the toxicity of Glu on the survival of control SMNs and SMNs lacking in TASK3 (another leak K^+ channel highly expressed in MNs). Conclusively, beneficial effects of Mit-A were almost fully absent in SMNs lacking in TASK1. In SMNs isolated from the SOD1^{G93A} mouse model of ALS both, Glu-induced alterations in $[Ca^{2+}]_i$ and Glu toxicity were amplified in comparison with non-transgenic SMNs. Noticeably, Mit-A also was beneficial for SMNs obtained from the ALS model. Altogether, these outcomes obtained in vitro support neuroprotective effects of Mit-A on MNs subjected to excitotoxic stimuli by a TASK1-dependent mechanism. Beneficial action of Mit-A on the in vitro ALS model signals this anti-cancer drug as a feasible therapeutic tool to minimize excitotoxic damage in a multitude of neuropathologies associated with a dysregulation of neuronal excitability and/or excitotoxic degeneration, such as ALS.

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Plycamycin, motoneuron, excitability, TASK1, neurodegeneration, ALS

O7-03

CHARACTERIZATION OF HISTOLOGICAL CHANGES IN OVARIES OF DIABETIC RATS

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Introduction and objectives: Goto-Kakizaki rats are an important model to study Diabetes Mellitus type II, nevertheless a chronic hyperglycemia is a problem that complicates the possibility of expanding its colonies showing poor fertility and a serious problem for experimental studies and of which these rats need. In previous studies, alterations have been shown in the astral cycle of diabetic rats contrary to those with a normoglycemic profile. We think that under this multi-organic matrix a functional alteration of the hypothalamus-pituitary-ovary axis must be found. Our objective in this study is to induce this possible altered effect in the hormonal cycle through the observation of the histology that present the ovaries of these animals after a long fertility cycle.

Methods and materials: Eight Goto-Kakizaki (diabetic) rats have been used and eight Wistar (normoglycemic) rats classified in two different poblational groups. In groups named “young rats” and another of “mature rats” matching their sacrifices among two months and fourteen months of life respectively. Once the sacrifice was done using hermetic cage and exchanging the normal atmosphere with a higher concentration of carbon dioxide, the ovaries were dissected and processed to be submitted to a process of a fixation inclusion of paraffin. Ten micrometer cuts of the anatomic pieces were stained with hematoxyline and were visualized via optic microscope. Moreover, the quantitative study has been described as the ovarian parenchyma architecture and qualitative data that could be highlighted when comparing the two strains of mentioned rodents.

Results: Both strains in young status rats did not show significant differences. However, between different age groups, alterations have been observed in the ovarian architecture due to ageing, being more emphasized in GK strain. A fourteen month group of old rats show differences both quantitatively and quantifiable inclusive indicating a possible major presence of anovulant cycles in diabetic GK rats.

Conclusions: Histology present in mature GK rats concord with studies which support the major presence of anovulant cycles in this diabetic rat group opposite to Wistar rats. We are marking the alterations in the studied variables (follicular fraction/estrus ratio and number of corpus luteum). Findings in situ of ovary histology allowing future open lines of investigation in the hypothalamus and pituitary as subsequent steps in the hypothalamus-pituitary-ovarian hormonal axis and in this way to explain the alterations in the gonadotropine hormone pulses and the astral cycles.

DM2, GK Rat, Ovary, Corpus Luteum

O7-04

IN VITRO AGING OF RAT HIPPOCAMPAL NEURONS IS ASSOCIATED TO CHANGES IN CALCIUM RESPONSES TO NMDA AND EXPRESSION OF IP3 RECEPTOR ISOFORMS

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Aging promotes cognitive loss and susceptibility to neurodegenerative disorders and both are strongly related to neuronal damage. Mechanisms of aging-dependent susceptibility to neuron damage are poorly known but they have been related to dishomeostasis of intracellular Ca^{2+} .

Accordingly we asked whether intracellular Ca^{2+} homeostasis and intracellular Ca^{2+} channels in neurons are remodelled during aging. For this end, we used calcium imaging in short-term and long-term cultures of rat hippocampal neurons represent young, mature and aged neurons. Rat hippocampal neurons culture for more than 20 days (aged neurons) display many of the typical hallmarks accompanying neuronal aging *in vivo*. Specifically, we studied calcium responses to glutamate receptor agonist N-methyl D-aspartate (NMDA) in different time-cultured neurons. We also asked whether inositol-1,4,5-triphosphate receptors (IP3Rs) involved in Ca^{2+} release from Ca^{2+} stores at the endoplasmic reticulum (ER) change with the neuronal aging. For this end, expression of the three isoforms of the IP3Rs was tested using quantitative immunofluorescence. Consistently with previous results in neuronal aging, we found that NMDA increases cytosolic Ca^{2+} concentration in rat hippocampal neurons were small in young neurons cultured for only 6 days *in vitro* (6 DIV) and increased in mature (16 DIV) and aged neurons (24 DIV). We also found that hippocampal neurons in culture express all three IP3Rs isoforms in young, mature and aged neurons. In addition, expression of three isoforms change with time in culture. Specifically, expression of IP3R1 is low in young neurons and increases significantly in mature and aged neurons. Similar results were obtained with IP3R2. In contrast, IP3R3 is expressed to the same extent in young and mature neurons and increases significantly only in aged neurons. These results indicate that *in vitro* aging of hippocampal neurons is associated to changes in Ca^{2+} responses to NMDA and changes in expression of IP3R isoforms. This remodelling might contribute to cognitive decline in the elderly at the expense of increased susceptibility to neuron cell. This work was supported by a grant BFU2015-70131R from Ministerio de Economía y Competitividad, Spain to CV and LN.

Aging, Calcium, hippocampal neurons, NMDA, IP3Rs

O7-05

ACTIVATION OF VISCERAL INSULAR CORTEX DURING TASTE PROCESSING IN RATS: THE ROLE OF NOVELTY

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Despite the large number of studies about it, the role of the insular cortex in the processing of interoceptive stimuli is not clear yet. Several studies have demonstrated the role of the gustatory insular cortex in the processing of tastes, and, especially, in the recognition of the novelty of said tastes. However, there is little information that relates de insular visceral cortex with such processing. The present study aims to explore the role of viscera insular cortex in relation to the processing of gustatory neophobia, a response that occurs when an animal encounters a new taste for the first time and that attenuates with the passage of successive presentations. As an index of neural activation, the number of active c-Fos cells in adult Wistar rats was counted during the first presentation of a saccharin solution (Novel), the second exposure (Familiar 2) and the exposure on the sixth day (Familiar 6). In addition, it was compared with neural activation in the baseline with water. The results show an activation of the insular visceral cortex during the presentation of water in the baseline compared to the control area. Said activation was maintained with the passage of the presentations. The gustatory insular cortex showed greater activation than the control area on the Novel day, which is in line with previous investigations. The results obtained in the insular visceral cortex could point to a relationship between this structure and hydromineral processing that would need an exploration in detail.

Insular cortex, neophobia, c-Fos, taste recognition

O7-06

DEVELOPMENT OF MITRAL STENOSIS SCENARIOS IN THE SIMMAN MEDICAL SIMULATION MODEL. PHYSIOPATHOLOGY AND APPROACH OF POSTOPERATIVE COMPLICATIONS

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Introduction: We developed a simulation-based educational tool to teach medical students the natural history of mitral valve disease and its post-operative complications. Using the realistic SimMan manikin (Laerdal Inc.), we developed eight protocols leading to a problem based learning system that allow the acquisition of skills for clinical practice.

Objective: We aim to link professional clinicians with university educators to develop a proper environment to allow the exchange of information, including theory and practice. This scenario will serve to train students and residents to achieve educational competences.

Methods: 1) Cardiovascular surgeons wrote medical records based on real cases of left side valve diseases as mitral stenosis. 2) Critical steps during simulation were set, specifying physical exploration, diagnostic tests and treatments. SimMan scenarios have a realistic time course of vital signs and render many other physiological variables, such as “Arterial PO₂” or “Blood Pressure”. The time course and evolution of clinical cases were adjusted in a realistic manner by using the Human program. Within each scenario, students must reach a potential diagnosis analyzing EKG and hemodynamic conditions, to propose other diagnostic test or surgical treatment. Their decisions are constantly checked by questionnaires in conjunction with academic lessons on videos added to the flow chart. There is also integrated branch of different steps depending on students’ performance.

Results: At first, students had significant difficulties in successfully completing the protocols. However, after proper guidance, the results were gradually improving. Doctors incorporated important practical skills to support their teaching and university professors acted as coordinators, checking the implementation of the theoretical basis of the physiopathology to real clinical cases.

Conclusions: The use of a realistic manikin and the study of clinical cases help students to better understand key concepts in medicine as exploration, diagnosis and treatment of mitral valve disease. This scenario can be easily adapted to ECOE evaluation in the Spanish Medical System.

Mitral-Stenosis, Advanced-Teaching, Simulation; ECOE

Oral session 8: Neurodegeneration

O8-01

EMPAGLIFLOZIN IMPROVES COGNITIVE IMPAIRMENT IN A MIXED MURINE MODEL OF ALZHEIMER’S DISEASE AND TYPE 2 DIABETES

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Alzheimer's disease (AD) is the most common type of dementia, and although age remains the main risk factor to suffer AD, epidemiological studies support that type 2 diabetes (T2D) is a major contributor. However, the mechanisms underlying this relationship are not fully known. It is also important to bear in mind that AD does not have a successful treatment, making necessary to search for new therapeutic alternatives that can prevent, delay or treat the disease. Although with evident limitations, T2D has different therapeutic options. Among these, empagliflozin (EMP) is the most recently approved and commercialized inhibitor of the sodium-glucose transporter 2. In order to deepen in the close relationship between AD-T2D and new therapeutic opportunities, we have recently developed mixed murine model by crossing an AD mouse (APP/PS1) with a T2D mouse (db/db). We have characterized our APP/PS1xdb/db mice at metabolic and central level; at 4 weeks of age, before AD or T2D pathologies begin; at 14 weeks of age, when T2D is present but there is no AD pathology yet; and at 26 weeks of age, when both pathologies are fully established. Learning and memory are severely affected in APP/PS1xdb/db mice at early stages. Our AD-T2D model presents significant brain atrophy, increased hemorrhage burden and tau pathology. Surprisingly, we observed a shift in A β soluble/insoluble levels in our APP/PS1xdb/db mice, favoring more toxic soluble species. Moreover, in vivo and in real time assessment of cerebral amyloid angiopathy (CAA) by multiphoton microscopy, suggests an increment of CAA in the mixed colony. We have also treated our mice with EMP in the long term, and we have observed that learning and memory processes are improved after EMP treatment. Altogether our data show that the APP/PS1xdb/db mouse is a relevant tool study AD-T2D relationship. Moreover, EMP might be an appealing approach, as a safe and approved treatment, that may ameliorate cognitive decline associated to AD and T2D.

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AD, T2D, empagliflozin, APP/PS1, db/db, amyloid

O8-02

NEUROPROTECTIVE EFFECTS OF THE ANTICANCER ANTIBIOTIC MITHRAMYCIN IN TWO MODELS OF MOTONEURON DEGENERATION

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Despite their different clinical manifestations, neurodegenerative diseases are underpinned by a multitude of common factors. Regarding this issue, alterations in intrinsic membrane excitability may determine differential vulnerability to excitotoxicity of specific neuronal subpopulations in neurodegenerative disorders. Somatic motoneurons are the vulnerable subpopulation to amyotrophic lateral sclerosis (ALS); a fatal neurodegenerative disease coursing with hyperexcitability as one of the earliest manifestations in sporadic and familial ALS patients. Outstandingly, hyperexcitability and excitotoxicity are common pathogenic hallmarks in motoneuron degeneration models with different etiology, i.e. ALS and traumatic injury of a motor nerve. Unpublished results from our lab have demonstrated that upregulation of the transcription factor Sp1 (specificity

protein 1) contributed to motoneuron degeneration regardless of the causal origin, by modulating intrinsic membrane excitability and vulnerability of motoneurons to an excitotoxic agent. Then, we hypothesized that mithramycin A (Mit-A), a FDA-approved anticancer antibiotic that interferes with Sp1 binding to DNA, could be neuroprotective for the two models of motoneuron degeneration. Strikingly, two doses (30 or 300 $\mu\text{g}/\text{kg}/\text{d}$) of Mit-A, administered in the drinking water from the day in which nerve injury was inflicted, drastically reduced motoneuron loss at 21 days after 2-mm segment resection of the XIIth nerve. Furthermore, daily Mit-A administration (30 $\mu\text{g}/\text{kg}/\text{d}$) from P30 resulted neuroprotective for lumbar motoneurons in the transgenic SOD1^{G93A} mouse model of ALS. Accordingly, Mit-A delayed symptoms onset, alleviated weight loss, prolonged lifespan, and improved motor performance (rotarod, footprint and runtime tests) of SOD1^{G93A} mice relative to the vehicle-treated group. The occurrence of fibrillations is indicative of myocytes denervation, a characteristic hallmark of ALS revealed with needle electromyography. Thus, the frequency of fibrillations gradually increased from week 13 to week 16 of age in transgenic mice whereas these were generally absent in their non-transgenic littermates. Interestingly, Mit-A treatment drastically decreased the rate of fibrillations at all ages tested. Altogether, these results indicate that Sp1 is also a key partner in an overall mechanism contributing to motoneuron degeneration and point to Mit-A as a neuroprotective agent with therapeutic interest for the treatment of diverse motor pathologies regardless the causal origin.

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Motoneurons, neurodegeneration, neurotmesis, amyotrophic lateral sclerosis, Sp1

O8-03

NEUROPROTECTIVE EFFECTS OF p11 DOWN-EXPRESSION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Excitotoxicity is a major pathogenic mechanism that contributes to motor neuron (MN) degeneration in Amyotrophic Lateral Sclerosis (ALS). In this line, MN hyperexcitability, resulting from an increase in intrinsic membrane excitability (IME), determines MN vulnerability to excitotoxicity, mainly by exacerbating the intracellular Ca²⁺ mobilization triggered by excitatory neurotransmitters, thus leading to loss of neuronal function and, subsequent cell death. Unpublished results from our group indicate that p11, which hampers expression of the leak K⁺ channel TASK1 at the plasma membrane, determines IME and vulnerability to excitotoxicity of MNs. So, reducing p11 expression might be a reasonable strategy against excitotoxicity, since it would promote TASK1 expression at cell surface and the subsequent reduction of IME and vulnerability of MNs. In this context, we hypothesize here a neuroprotective action of p11 down-expression in the SOD1^{G93A} mouse model of ALS. Addition of a small interfering RNA directed against p11 (siRNAp11) to primary cultures of spinal cord MNs (SMNs), drastically reduced both, IME and toxic effects of glutamate (150 μM , 30 min) on SMNs via TASK1, as compared with a non-targeting RNA (cRNA). Interestingly, siRNAp11 also attenuated hyperexcitability and vulnerability to glutamate of SMNs isolated from SOD1^{G93A} embryos (SMNs^{G93A}). Ca²⁺ imaging from SMNs revealed that, siRNAp11, via TASK1, reduced baseline intracellular Ca²⁺ concentration ([Ca²⁺]_i), attenuated glutamate-induced rapid increase in [Ca²⁺]_i, and delayed [Ca²⁺]_i deregulation (indicative of

impending neuronal death) evoked by the excitotoxic insult. siRNAP11 produced similar effects on Ca^{2+} dynamics in SMNs^{G93A}. Finally, we assessed feasible neuroprotective effects of siRNAP11 on the ALS murine model. Chronic administration of siRNAP11 to SOD1^{G93A} mice, beginning at a pre-symptomatic stage (2-month-old), delayed both, motoneuron loss in the lumbar spinal cord, and symptoms onset. It also alleviated weight loss, improved motor performance (rotarod, footprint, runtime, and grip strength), and prolonged lifespan of SOD1^{G93A} mice, as compared with cRNA-treated transgenic animals. Altogether, these outcomes indicate that p11 is a potential therapeutic target for the treatment of ALS and, maybe for other neuropathological conditions which course with hyperexcitability and excitotoxicity as pathogenic mechanisms.

ALS, motoneurons, intrinsic excitability, p11

O8-04

APOLIPOPROTEIN D PROMOTES CELL SURVIVAL AND LYOSOMAL MEMBRANE INTEGRITY IN NIEMANN-PICK TYPE A DISEASE

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Damage to cell membranes is common to many neurodegenerative diseases. Lysosomes are particularly sensitive to membrane permeabilization because intraluminal acidic pH and stable membrane-dependent proton gradients are required for their function. Lysosomal impairment gives rise to Lysosomal Storage Diseases (LSDs), genetic, early onset, neurodegenerative diseases that result in poor survival and both systemic and nervous system dysfunction. All LSDs are associated to leukodystrophy or myelination problems. Apolipoprotein D (ApoD) is essential for the maintenance of lysosomal functional integrity in glial cells. It ensures processes as diverse as cell survival upon oxidative stress (by reverting membrane permeabilization and loss of pH gradients), adequate compaction of myelin (by controlling glycolipid recycling processes), or proper phagocytic activity after nervous system injury. The crucial role of ApoD within the lysosome led us to study the potential effects of ApoD on a particularly devastating LSD, the Niemann Pick type A disease (NPA), caused by loss of function mutations in the acid sphingomyelinase gene, which results in sphingomyelin accumulation in lysosomal and plasma membranes. NPA patients rapidly develop progressive neurodegeneration, cerebral and cerebellar atrophy, significant Purkinje cell loss, and myelin deficiencies. No treatment is available today, in spite of various attempts with pharmacological, enzyme-replacement, or cell-based strategies. Using two independent NPA-patient derived fibroblasts cell lines and two healthy control lines, we demonstrate that, as in glial cells and neurons, ApoD is targeted to lysosomes of NPA fibroblasts. Oxidative stress induces an accelerated entry of ApoD into the lysosomal compartment of healthy cells. However, such accelerated targeting is lost in the diseased cells, contributing to the vulnerability of NPA lysosomes. We assessed lysosomal functional integrity and cell survival, using L-leucyl-L-leucine methyl ester as positive control for lysosomal membrane rupture, and pre-treatment of healthy control cells with sphingomyelin as a phenocopy of NPA disease. By measuring cathepsin B activity, galectin-3 subcellular location, and lysosomal pH measures, we demonstrate that exogenously added ApoD is able to significantly reduce lysosomal permeabilization and NPA-promoted lysosomal alkalization. ApoD addition reverts the accumulation of oxidized products in lysosomes and of

lipid peroxidation in NPA cells, resulting in a significant increase in cell survival. ApoD protection of lysosomal integrity is able to counteract biological deterioration in NPA cells, and open therapeutic opportunities for this devastating disease.

Lysosomal storage disease, Demyelination, Lipid peroxidation

O8-05

ALTERATIONS IN THE DYNAMICS OF CENTRAL SYNAPSE IN MICE CARRYING THE HERC 1 UBIQUITIN LIGASE PROTEIN MUTATION

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The mutation *tambaleante* (*tbl*) is caused by a Gly483Glu amino acid substitution in the HERC1 E3 Ubiquitin ligase protein. This change causes in adult mice an ataxic syndrome due to cerebellar Purkinje cell death. The phenotype of *tbl* is more complex than initially described. Thus, defects of peripheral myelin, and impairment of motor performance and associative learning have also been described. *Herc1* mutations have also been described in humans, showing a polymorphic syndrome with different neurodegenerative signs, in which the intellectual disability is the common symptom. Synaptic transmission is essential for any cognitive event. Therefore, we have analyzed the main synaptic vesicles dynamics by using cultures of hippocampal neurons, and immunocytochemical analysis of MAP2, SV2B and clathrin. The characterization of synaptic vesicle pools by destaining experiments of FM1-43, revealed that all synaptic vesicle pools were decreased in *tbl* mutation almost by half. We quantify the ready releasable pool (RRP) and the resting pool (RP), which are suitable for quantification with this technique. MAP2 expression is clearly diminished in *tbl* neurons respecting control ones ($p < 0.01$). In the same way, immunoreactivities for SV2B and clathrin are consistently lesser in *tbl* than in control cultured hippocampal neurons ($p < 0.005$ in both cases). Altogether, present results show that the overexpression of mutated HERC 1 elicits changes in the synaptic vesicle dynamic such are: (i) reduced RRP and RP synaptic vesicle pools; (ii) the diminution in the total number of synaptic vesicles expressing SV2B vesicular marker; and, (iii) a possible alteration on the clathrin mediated endocytosis. The overexpression of mutated HERC1 protein deregulates the ubiquitin-proteasome system, increasing the autophagy which was proposed as the basis of the neuropathological changes found in *tbl* mice. Our results open new possibilities on the malfunction of the overexpressed protein. HERC1 acts in the formation of ternary complex between the heavy chain of clathrin and the ARF and Rab GTPases families, and could play a key role in the endocytic processes. Thus, the deregulation of the endocytic process at the presynaptic terminal could alter the normal synaptic vesicle recycling, and it could be hypothesized that this alteration is the responsible for the reduction on the number of synaptic vesicles.

Synaptic-transmission, synaptic-vesicles, endocytosis, HERC1, *tambaleante*

O8-06

HISTONE DEACETYLATION IN HUNTINGTON'S DISEASE

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Transcriptional dysregulation in Huntington's disease (HD) is an early event that affects the expression of genes involved in survival and neuronal functioning. Epigenetic dysregulation has offered an attractive hypothesis to explain the coordinated disruption of multiple genes and the reversion of both molecular and phenotypic traits after treatment with chromatin-modifying drugs at the preclinical stage. Increasing evidence has accumulated in recent years by enlarging the catalogue of DNA and histone covalent modifications that are altered in animal and cellular models, and human postmortem brains. Our research has focused on deacetylation of histone H3, and its involvement in gene downregulation in HD. In the last few years we have examined the gene expression and histone H3 acetylation patterns across the brains of two transgenic mouse models of HD, the R6/1 and N171-82Q strains, at different stages of the pathology. Whereas genome-wide approaches showed that defective gene expression and histone deacetylation did not fully coincide in the hippocampus of symptomatic mice, the meta-analysis with publicly available HD transcriptomes (including datasets from other brain areas and mouse models) suggested that histone deacetylation may converge with additional tissue and/or temporal factors to promote/label an effective transcriptional failure. To get further insights, we have recently analyzed these mice at prodromal stages before overt pathological phenotype. The transcriptional signatures in the animal brains contained a strong tissue-specific component and were potentially linked with the defective activity of several chromatin-modifying factors. However, we only detected an incipient decrease in the protein levels for the lysine acetyltransferase CREB-binding protein (CBP), concomitant with highly selective deacetylation events. Although histone deacetylation may associate with genes susceptible to downregulation, other epigenetic impairments may exert an indirect influence over transcriptional dysregulation in HD. Based on these studies, we propose that the neuronal epigenetic niche is compromised in early stages of HD, leading to an altered transcriptional programme prominently involved in neuronal identity, which worsens during the progression of the disease.

Polyglutamine, transcription, epigenetics

Oral session 9: Gastrointestinal

O9-01

GLUCOCORTICOIDS AFFECT FXR/FGF21/FGF19-MEDIATED CROSSTALK BETWEEN LIVER AND INTESTINE

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Background and aim: Fibroblast growth factor (FGF) 19 (Fgf15 in rodents) and FGF21, which are secreted by the ileum and the liver,

respectively, play an important role in the crosstalk between both organs to regulate metabolism during feeding/fasting periods. FGF19, whose expression is upregulated by the bile acid (BA) sensor FXR, controls BA synthesis by downregulating the key enzyme, cholesterol 7 α -hydroxylase (CYP7A1). In previous studies, we have reported that glucocorticoids (GC) disrupt this control by reducing FGF19 expression without increasing CYP7A1 levels, but it remains unknown their impact on FGF21 expression and BA homeostasis. Therefore, the aim of this study was to evaluate the ability of GC to interact with FXR/FGF19/FGF21-mediated ileum-liver crosstalk and the underlying mechanism of CYP7A1 regulation by FGF21.

Methods: In vivo studies were carried out using C57BL/6 mice that received (i.p.) dexamethasone (DEX) 0.25-5 mg /kg/day for 7 days. In silico analysis of the CYP7A1 promoter (prCYP7A1) was performed to detect putative response elements for downstream effectors of FGF21 pathway. To evaluate prCYP7A1 activation, changes in luciferase activity were determined in human hepatoblastoma HepG2 cells co-transfected with plasmids containing human FGF21 ORF and prCYP7A1 (wild-type or mutated) with IRES-EGFP or Luc2 sequences, respectively. In a separate set of experiments, cells were incubated with medium containing pure human recombinant FGF21 (1.1 μ g/ml) or with medium conditioned for 24 h by cells transfected with prCMV-human FGF21.

Results: In mice, at non-hepatotoxic dose (0.5 mg/kg b.w./day for 7 days), GC induced ileal Fgf15 down-regulation and liver Fgf21 up-regulation, without affecting Fxr expression. Fgf21 mRNA levels correlated with these of several genes involved in glucose and BA metabolism such as Cyp7a1 and BA-conjugating enzyme Baat. Moreover, in vitro experiments revealed that over-expression of FGF21 in HepG2 cells inhibited prCYP7A1 activity. This inhibition was mimicked by incubation with culture medium previously conditioned by FGF21 over-expressing cells or with pure human FGF21 protein. In silico study showed the presence of a putative response element for activated effectors of FGF21 pathway in prCYP7A1 sequence. However, deletion of this response element in prCYP7A1 did not abolish FGF21-mediated CYP7A1 downregulation.

Conclusion: Owing to alterations in both the FXR/FGF19-mediated ileal control of CYP7A1 expression and the secretion of FGF21 by the liver, which inhibits CYP7A1 promoter through an autocrine mechanism, the therapy with GC may interfere with the regulation of hepatic BA synthesis and therefore affect the control of cholesterol metabolism.

FGF21, CYP7A1, Autocrine, Bile Acid, Glucocorticoids

O9-02

IMPLICATION OF SIGMA 1 RECEPTORS IN THE DEVELOPMENT OF INFLAMMATION-ASSOCIATED VISCERAL AND SOMATIC HYPERSENSITIVITY IN MICE

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Background and aims: Intestinal inflammation is associated to both visceral and somatic hypersensitivity. Sigma-1 receptors (σ 1Rs) modulate nociception and pain sensitization and might be important in the development of inflammatory states. We assessed the role of σ 1Rs on colitis-associated changes in somatic and visceral sensitivity using a murine model knockout for σ 1Rs.

Methods: Adult CD-1 wild-type (WT; n=24) and σ 1R knockout (σ 1R KO; n=27) male mice were used. Colitis was induced by exposure to a

3% solution of dextran sodium sulfate (DSS) during a 5-day period (experimental days 0 to 5), followed by a 2-day recovery. A von Frey test was used to assess visceral and referred somatic mechanosensitivity (abdominal and plantar withdrawal responses, respectively). Changes in mechanosensitivity were assessed before (experimental day -1), during (experimental day 3) and after colitis induction (experimental day 7). At termination, expression of immune and nociceptive markers (RT-qPCR, Western blot) was assessed in colon and lumbosacral spinal cord. **Results:** σ 1R KO mice showed attenuated clinical signs and colonic inflammation, as assessed macro and microscopically. Visceral and somatic mechanosensitivity was similar in WT and σ 1R KO mice before the induction of colitis (basal conditions). In WT mice, colitis was associated to a time-related development of somatic and visceral mechanical hypersensitivity, revealed as a reduction in pain thresholds in the von Frey test. In σ 1R KO neither somatic nor visceral mechanical sensitivity was affected during inflammation. Basal expression of colonic nociceptive markers was similar in WT and σ 1R KO mice. However, in σ 1R KO, CB1 and PAR-2 were up-regulated while TRPV1 was down-regulated ($P < 0.05$ in all cases). During colitis, regardless the phenotype considered, an overall down-regulation of colonic nociceptive markers was observed. At spinal level, neither gene expression of sensory receptors (GluR2, MOR, NK1, NR2B) nor the level of proteins involved in sensitization mechanisms (ERK/pERK, CamKII/pCamKII, P38/pP38 and GFAP) were affected.

Conclusions: σ 1R are involved in intestinal inflammation and the development of inflammation-associated visceral and somatic hypersensitivity. From the present results, the underlying mechanisms mediating these functional responses cannot be inferred.

Colitis, hypersensitivity, pain, sigma 1 receptors

09-03

LACK OF REELIN REPRESSES P53 EXPRESSION IN INFLAMED MICE COLON

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Reelin is an extracellular matrix protein that regulates the migration of neurons during brain development⁽¹⁾. We have previously reported that: i) reelin expression is up-regulated by acute colitis and down-regulated in colon adenocarcinoma^(2,3) and, ii) reeler mice (lack of reelin) are more susceptible to develop both, colitis and colon tumours^(2,4). As chronic inflammation abolishes the action of the p53, a tumor suppressor protein⁽⁵⁾, the goal of this study was to determine whether p53 mediates the protective-role assigned to reelin in inflammation and colon cancer development. Colonic inflammation was induced in 3 month-old wild-type and reeler mice by 9 days oral administration of 3% dextran sulfate sodium (DSS) in the drinking water. The p53 mRNA relative abundance (real-time PCR), its protein abundance (western-blot) and cell localization (immunohistochemistry) were determined in the distal colon of either DSS-treated or untreated mice. In wild-type mice, as previously observed with reelin2, the 9 days DSS-induced colon inflammation increases p53 mRNA levels by a factor of 4.41 (± 0.9) as compared with the non-inflamed distal colon. The p53 mRNA levels measured in the colon of DSS-untreated reeler mice are similar to those found in the wild-type mice but inflammation does not modify p53 mRNA abundance. As with p53 mRNA measurements, p53 protein abundance is similar in non-inflamed colon of wild-type and reeler mice but it was significantly increased in the inflamed colon of wild-type mice. DSS-treatment has no effect on the p53 protein abundance in the reeler mice. The immunohistochemistry assays corroborate these results. In DSS-untreated mice colon, a weak p53 (either

unphosphorylated or phosphorylated) signal is observed in both, crypts and surface epithelial cells. DSS-treatment significantly increased p53 signal in the wild-type colon, but not in the reeler colon, being particularly abundant in the inflamed region. In conclusion, as far as we know, the results show for the first time that in the colon, reelin is needed to increase p53 expression (mRNA and protein) in response to inflammation and suggest that this could be one of the mechanisms by which reelin protects from inflammation transition to colon cancer.

⁽¹⁾ D'Arcangelo et al. (1995). *Nature*. 374:719-723; ⁽²⁾ Carvajal et al. (2017) *Biochim Biophys Acta*.1863(2):462-473; ⁽³⁾ Serrano-Morales et al. (2017) *Mol Carcinog*. 56(2):712-721; ⁽⁴⁾ Carvajal et al. (2017) *Biochim Biophys Acta*.1863(9):2126–2134; ⁽⁵⁾ Levine (2011) *Genes Cancer*. 2(4): 503–516.

p53, reelin, inflammation, cancer, colon

09-04

GENDER-RELATED DIFFERENCES IN COLONIC MECHANICAL SENSITIVITY IN RATS

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Background & aims: Controversy exists concerning the influence of gender on pain sensitivity in animals and humans. Although some evidences suggest the existence of sex-related differences in visceral sensitivity, this phenomenon has not been studied in detail. In this work, we assessed gender-related differences in colonic mechanosensitivity in rats. **Methods:** Adult, female and male SD rats were used. Colonic sensitivity was assessed by determining pain-related visceromotor responses (VMR) to isobaric colorectal distension (CRD) with a barostat. Two protocols of isobaric colonic phasic distensions were used: i) ascending phasic distensions (10 to 80 mmHg, at 10 mmHg steps, with a pulse duration of 30 s and a 5 min interval between pulses) and, ii) repetitive phasic distensions (12 pulses at 80 mmHg, with a pulse duration of 30 s at 5 min intervals). The ascending phasic protocol served to determine pain thresholds and the repetitive phasic protocol to assess the development of mechanical sensitization. Data are mean \pm SEM.

Results: Visceromotor responses to ascending phasic isobaric distension of the colon were similar in males and females, with similar pain thresholds (males: 24 \pm 2.2 mmHg, n=10; females 30 \pm 4.8 mmHg, n=13). During the repetitive phasic protocol initial VMR (1st distension) were similar in males (0.30 \pm 0.03, n=20) and females (0.31 \pm 0.04, n=27). Both sexes showed acute mechanical sensitization, as indicated by an increase in the VMR from the 1st to the 12th distension (VMR 12th distension; males: 0.97 \pm 0.07; females: 0.47 \pm 0.05; $P < 0.05$ vs. respective VMR during 1st distension). Sensitization responses were significantly higher in males than in females (percent change from 1st to 12th distension; females: 83.89 \pm 17.44 %; males: 256.20 \pm 27.39 %; $P < 0.05$). Accordingly, overall responses to CRD were significantly higher in male (AUC distensions 1 to 12: 6.95 \pm 0.5) than in female rats (AUC distensions 1 to 12: 4.56 \pm 0.40; $P < 0.05$ vs. males). No differences in VMR were observed within animals when the distension protocols were repeated over time.

Conclusions: Results obtained indicate that visceral (colonic) mechanical sensitivity and pain thresholds to mechanical stimulation are similar in female and male rats. However, males show higher responses during acute mechanical sensitization, suggesting the existence of gender-related differences in the development of visceral hypersensitivity. The gender has to be considered when addressing pain sensitization (hyperalgesia and/or allodynia) in animal studies.

Rat, gender, hypersensitivity, visceral pain

O9-05

DAB-2 PROTEIN IS UPREGULATED AND COLOCALIZED WITH E-CADHERIN AT JUNCTIONS IN MICE COLON INFLAMMATION

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Dab2 is an intracellular adaptor protein involved in clathrin-mediated endocytosis in the kidney, visceral endoderm and intestine⁽¹⁾. We previously proposed that Dab2 mediates the intestinal endocytosis of milk macromolecules in the suckling rats^(2,3) and that is involved in apical junction establishment in Caco-2 cells⁽⁴⁾. In the current study we investigate whether: i) inflammation affects intestinal Dab2 expression and ii) Dab2 participates in the maintenance of cell-cell junctions during this pathology. Colon inflammation was induced in 3 month-old mice by oral administration of 3% dextran sulfate sodium (DSS) in the drinking water during 3, 6 or 9 days. The Dab2 mRNA abundance (RT-PCR) and the cell localization of Dab2, E-cadherin and Zonula Occludens-1 (ZO-1) proteins (immunofluorescence) were determined in the distal colon of DSS-untreated and treated mice. DSS-induced colon inflammation was confirmed by the increase of IL-1 β mRNA abundance. 9 days DSS-treatment increases Dab2 mRNA levels by 7 ± 0.9 times when compared with the normal distal colon. 3 days DSS-treatment increases the Dab2 protein abundance in the crypt cells and colonocytes and its co-localization with E-cadherin at the cell to cell junctions. As inflammation progresses, the normal structure of the colon epithelium is destroyed and the expression of the cell-cell junctions proteins (E-cadherin and ZO-1) is reduced by 6 days DSS-treatment, being absent following 9 days treatment. Dab2 co-localizes with ZO-1 neither in control nor in DSS-treated colon. In conclusion, as far as we know, this is the first report showing that epithelial cells from mouse distal colon responds to DSS-colitis by increasing Dab2 expression and the results suggest that Dab2 might exert an anti-inflammatory action by recruiting E-cadherin to the adherens junctions and thus contributing to the junction stability at the early stages of inflammation.

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⁽²⁾ Vázquez-Carretero et al. (2011) *J Cell Biochem* 112:354–361. ⁽³⁾

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Colon, Dab2, E-cadherin, ZO-1, inflammation, junctions

O9-06

OVERSTIMULATION OF COLONIC TLR-4 WITH LPS DOES NOT INDUCE VISCERAL HYPERSENSITIVITY IN RATS

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Background & aims: States of dysbiosis of the gut commensal microbiota and altered visceral (intestinal) sensitivity are common findings in several gastrointestinal disorders. Although a causal relationship has not been established, evidences suggest that altered host:microbial interactions during dysbiosis might be important in the development of visceral hypersensitivity. Changes in Toll-Like Receptors (TLR)-dependent host:microbial interactions have been implicated in the intestinal responses to dysbiosis. Here, we assessed the potential involvement of TLR-4 in the development of colonic hypersensitivity in rats.

Methods: Adult, female SD rats were used. LPS (E. Coli 055:B5) was used to selectively overstimulate TLR-4. Effects of local

(intracolonic, 0.5 mg/rat, acute or repeated 5-day treatment, n=7 for each) and systemic (0.5 mg/rat, intraperitoneal, n=7) LPS on colonic sensitivity were assessed. Colonic sensitivity was assessed by determining visceromotor responses (VMR) to repetitive isobaric colorectal distension (CRD) with a barostat (2 sets of 3 pulses at 10 mmHg and 3 min duration, with a 30 min interval between sets). Data are mean \pm SEM.

Results: After local stimulation of colonic TLR-4 with intracolonic LPS a minor body weight loss (by 1%) was observed at 24 h post-administration, without other clinical signs. Basal VMR were similar in all animals. Neither acute nor repetitive overstimulation of colonic TLR-4 with intracolonic LPS affected VMR (Acute treatment: LPS, 0.87 ± 0.14 ; Vehicle, 0.79 ± 0.05 . 5-day repetitive treatment: LPS, 1.04 ± 0.11 ; Vehicle: 0.90 ± 0.13 . $P > 0.05$ in all cases). Animals treated with systemic (intraperitoneal) LPS had a loss of body weight (by 5 % at 24 and 48 h post-administration; $P < 0.05$) and a transitory drop of body temperature (by 2 °C within 10 h post-administration; $P < 0.05$). Nevertheless, no changes in colonic sensitivity during CRD were observed in the same animals (VMR at 24 h post-administration: LPS, 0.81 ± 0.13 ; Vehicle, 0.82 ± 0.28 ; $P > 0.05$).

Conclusions: These data indicate that selective TLR-4 overstimulation is not sufficient to induce visceral hypersensitivity in rats. Nevertheless, these observations do not exclude a role TLR-4-mediated signalling in the development of visceral hypersensitivity during dysbiosis. In dysbiotic states, several concurrent mechanisms elicited by changes in host:microbial interactions might be necessary for the development of intestinal hypersensitivity.

TLR-4, LPS, hypersensitivity, pain, host:microbial interactions

Oral session 10: Teaching & SECF Awards

O10-01

FLIPPED CLASSROOM METHOD, USING CONCEPT MAPPING STRATEGY IN AN ENDOCRINOLOGY COURSE

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The learning strategies of flipped classroom and concept mapping have been assessed as part of a repertoire of ideas to confront the increasing complexity of providing the total learning material in the classroom. The flipped classroom concept consists in the acquisition of knowledge outside the classroom using information made available by the educator on a web site. Effective learning can also be achieved by constructing concept mappings, a scholarly tool that enables the integration of information in the form of categories and generalities that stimulate critical thinking and analysis of the learned material. A benefit of the flipped classroom and concept mapping methods is the ability of the educator to transform the passive nature of a lecture into a collective and dynamic activity of students motivated by a new form of communication. Flipped classroom and concept mapping require a direct and active participation of students in the classroom. Generating responsibility in the student is a requirement that transcends knowledge and that must be associated with the complementarity of effectiveness and above all, ethics. Endocrinology is an optional subject in the last course of Biochemistry's Degree. This subject has been chosen to introduce concept mapping combined with the flipped classroom approach. The number of students is small and they have already acquired most of the basic concepts in Physiology. It maintains a web site to permit access of students with its general organization, laboratory lectures

and lecture-based information. During the present academic year, lectures (one and a half hour) were organized to enable students to work cooperatively in developing a specific concept mapping for each program's lessons. On the subsequent day, students were asked to present in the classroom the already constructed concept mapping for the corresponding topic and answer one or two related questions. All of the students obtained good grades in the final test (75% A or A+ and 25% B+). Finally, a survey was made to have a feedback for the new teaching experience. The results showed that 67% of the students regarded the learning experience as very good, 28% as good, and 3.5% as not entirely satisfied. No student regarded the experience as completely dissatisfied. We conclude that the flipped classroom and concept mapping practice strengthen our work of educators by stimulating communication, creating a learning community and providing an incentive to exploration and integration of the new knowledge beyond the classroom or the informatics experience.

Flipped classroom, concept mapping, cooperative learning

O10-02

ANTHROPOMETRY: AN EDUCATIONAL SCENARIO TO IMPROVE KNOWLEDGE IN PHYSIOLOGY

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Introduction: Anthropometry is defined as the study of evaluation of human body in terms of dimensions of bone, muscle and adipose tissue. In the last years, scientific literature has shown that traditional measurements are associated with certain biases in body composition.

Aim: Our objective was to teach how to measure body composition through a caliper and Bioelectrical Impedance Analysis to our “Physiology of Vegetative and Reproductive Functions” students who were studying their Degree in Biology.

Methods: Participants were 52 undergraduates who were grouped into 5 groups, these groups had different objectives during the semester. Basal Metabolic Rate and Body Composition were the main topics our students worked and subsequently they focused in concepts like fat mass, lean mass, skinfolds and waist-hip ratio. Students measured their anthropometric variables (through a scale and a caliper) and shared their own values, anonymously. To share data students used Google Docs in order each group could work their own objectives. Finally, every group had to write a scientific manuscript and expose to the others. Moreover, students were evaluated to elucidate if they learnt the concepts they worked. **Results:** Students showed they gained new concepts after our educational intervention, they learnt new tools like statistical methods or productivity apps (Google Docs) for research and they developed new skills such as data collection, results discussion and writing scientific manuscripts. Eventually, they reported satisfaction with the scenario we created for our subject.

Conclusion: Our educational scenario, starting from the concepts of Body Composition and Basal Metabolic Rate and framed in Anthropometry improved knowledge and skills in our students at the end of the semester.

Acknowledgements: This research was supported by Junta de Extremadura (Fondos FEDER – GR18040).

Anthropometry, undergraduates, university, education

O10-3

ROLE OF TYPE 2 DIABETES MELLITUS IN CENTRAL NEURODEGENERATION AND VASCULAR DAMAGE: IMPLICATION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common cause of dementia and has no successful treatment. Vascular Dementia (VaD) is the second common cause of dementia and the borderlines between AD and VaD are blurred in many cases. While age remains the main risk factor to suffer AD, in the last few years epidemiological and clinical studies have identified different metabolic alterations as risks factors to develop AD. Following this idea diabetes mellitus (DM) seems to play a relevant role at this level. Nevertheless, basic science studies are limited and the underlying mechanisms for this relationship (AD-DM) remain largely unknown. In order to explore the close relationship between both pathologies, we have developed a new model by crossing APP/PS1 mice with db/db mice (APP/PS1xdbdb). We characterized metabolic and cognitive evolution, before T2D or AD pathology are present (4 weeks), when T2D has debuted but no senile plaques are present (14 weeks) and when both pathologies are well established (26 weeks). APP/PS1xdbdb mice showed an age-dependent synergistic effect between T2D and AD. Significant brain atrophy and tau pathology were detected in the cortex by 14 weeks, that spread to the hippocampus by 26 weeks of age. Severe cognitive impairment was also detected as soon as at 14 weeks of age. Interestingly, in APP/PS1xdb/db mice we observed a shift in A β soluble/insoluble levels, and whereas more toxic soluble species were favoured, senile plaque were reduced. An overall increase of microglia activation was observed in APP/PS1xdb/db mice. We also found an early affection of metabolic parameters in APP/PS1xdb/db mice suggesting a two-way cross talk between both pathologies. In addition, at a cellular level a significant increase of neurite curvature was observed in prediabetic APP/PS1 mice, and this effect was worsened in APP/PS1xdbdb animals. Synaptic density, analysed by array tomography, was reduced in APP/PS1xdb/db mice whereas an intermediate state was observed, once more, in prediabetic-AD mice. Moreover, metabolic parameters predicted many of these alterations, supporting a role for T2D in AD pathology. Altogether, our data support the relevant role that metabolic alterations play at central level and help to elucidate the implication of diabetes in AD development. This model provide a relevant tool to further explore the relationship between T2D, AD and vascular implications, offering the possibility to assess therapeutic approaches that by improving metabolic control could delay or prevent AD pathology.

Alzheimer's disease, vascular dementia, type-2 diabetes

O10-4

INCREASED INTEREST IN SCIENCE AND PHYSIOLOGY AMONG STUDENTS OF SECONDARY EDUCATION AFTER THE OUTREACH ACTIVITY: “FROM DNA TO FUNCTIONAL TISSUE”

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With the final goal of promoting the dissemination of Physiology and to motivate students of Secondary Education to study Science, we organized the outreach activity: “From DNA to Functional Tissue”, at the School of Biology, University of Seville, Spain. During the activity, the students had the opportunity of 1) extracting their own DNA with a saliva sample, 2) visualizing histology slides from different human tissues and cells undergoing mitosis, 3) working with anatomical models, and 4) studying the different physiological adaptations to exercise. After the laboratory activities, the students filled a questionnaire and took part in a contest designed to evaluate the comprehension and strengthening of the information learned. With these activities, we expected to give the students a broader and integral vision of the human body from the molecule, to the cell, to the functional tissue, up to the whole organism. We hoped that the participants would consolidate better the information by this hands-on teaching approach, and would achieve a better understanding of the presence of physiology in daily life. After analyzing the data obtained from the competition results and the survey, we conclude that the students had understood and consolidated the information, and that the activity helped them to gain a higher degree of awareness of the importance of Science to our society. Besides, the easy experiments carried out by the students promoted their curiosity and encouraged them to learn about the functioning of our body, so the activity fulfilled our objective of inspiring more students to study science and physiology.

Outreach activities, Obligatory Secondary Education, Physiology

O10-5

AUGMENTED REALITY IN PHYSIOLOGY: A MORE REALISTIC VISION WITHIN VIRTUALITY

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Augmented reality (AR) is becoming one of the emerging technologies with a close penetration in university education, which aims to integrate content and virtual data with the physical environment in real time. Within the curricular area of physiology, through a project of teaching innovation during the 2017-2018 academic year, the AR looked for the enrichment of practical knowledge to deepen understanding of specific topics within the context of a Human Physiology course. With this project we aimed to provide physiology students with a supportive and practical environment, in which could put into play all the theoretical knowledge acquired. Two work tools, HP Reveal (Aurasma) and Aumentaty Author, were used to create new resources or materials for AR-based learning, whose possibility of recall and assimilation is superior to that derived from the information provided through traditional teaching methods. The definition of AR fundamentals, implemented with examples that illustrate several physiological processes, delivered either in the form of practical sessions in two courses of Human Physiology (Human Physiology I & Human Physiology II) for second-year students of Medicine, or as training workshops for teachers of different curricular areas, allowed them to familiarize with the terminology, tools and technology required to start experimenting with AR and helped them to become highly skilled users. Upon completion of each physiology course or teacher-training workshop, surveys were conducted to assess the perceived usefulness, pedagogical potential and continuity of the AR as a new didactic possibility to carry out teaching-learning. The results obtained reveal great acceptance of the AR technology by the students, emphasizing its easy use in practical and theoretical classes via their own mobile devices and a favorable positive attitude to use and incorporate the AR as a new resource that

helps them to visualize and understand, in real time, physiological processes, mechanisms and concepts otherwise difficult to assimilate in the classroom. Both, students and teachers shared the same concern for the use of AR, valuing very positively the experience. Therefore, under clearly defined objectives, the implementation of the AR would confirm that it is an excellent support strategy to complement the traditional teaching in physiology due to its high degree of interaction, being also easily transferable the adaptation of this knowledge to the rest of curricular areas.

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Augmented reality, emerging technology, interaction.

POSTERS

Poster session 1: Cell & Molecular, Sports, Teaching, Neurophysiology.

P1-01

LIPID RAFT-INTEGRATED PROTEIN MARKERS IN EXOSOMES. ASSOCIATION WITH PROTEIN SORTING IN NEURODEGENERATIVE DISEASES

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Exosomes are small membrane nanovesicles, generally 50 to 90 nm size, secreted by cells upon fusion with the cell membrane. When released, exosomes can propagate molecular content to other cells in long distances. In neurons, these nanovesicles contribute to regulating neuronal development, plasticity and regeneration. Related to neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson disease (PD), exosomes importantly contribute to propagating neurotoxic aberrant protein aggregates and misfolded markers, thus contributing to increase the neurotoxicity impact. Recent evidence has demonstrated that some of the typical hallmarks of AD and PD, i.e. amyloid- β peptide ($A\beta$) and α -synuclein (α -Syn) associate with lipid markers integrated in lipid raft membrane microdomains as part of their mechanisms of pathological self-aggregation. Lipid rafts are laterally organized structures within cell membranes based on a particular lipid composition enriched in gangliosides, sphingolipids and cholesterol. Our previous work has extensively demonstrated that these membrane microstructures are involved in AD, PD and other synucleopathies. Indeed, different lipid species such as ganglioside GM1 and cholesterol highly enriched in lipid rafts known to enhance $A\beta$ and α -Syn oligomerization are also involved in the propagation of toxic aggregates. We demonstrate here, using human neuroblastoma SHSY-5Y cells, that $A\beta$ and α -Syn markers are abundantly secreted specifically in 30-50 nm size vesicles. Other protein raft markers known to participate in AD pathology, such as flotillin-1 and the voltage dependent anion channel (VDAC) are also found in these exosomes. Noticeably, these <50 nm subclass shows a high content of ganglioside GM1, as an indicative of the potential involvement of this lipid class in toxic protein markers propagation. These experiments were performed by using SHSY-5Y cells previously differentiated with retinoic acid and Phorbol 12-myristate 13-acetate. SHSY-5Y cells were processed for immunofluorescence and confocal microscopy. Further analyses were performed by immunogold and transmission electron microscopy using specific antibodies

directed to the different protein markers. Overall, these results suggest that lipid rafts are involved not only in the mechanisms of conformational transition and oligomerization of toxic protein markers but also in the cell-cell propagation of aberrant molecular species that may enhance the neuropathological progression.

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Lipid rafts, exosomes, proteinopathies

P1-02

ROLE OF MELATONIN RECEPTORS IN THE SYNERGISTIC EFFECT OF MELATONIN ON CYTOTOXIC AND APOPTOTIC ACTIONS EVOKED BY CHEMOTHERAPEUTICS

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Background: Melatonin has antitumor activity via several mechanisms including its antiproliferative and proapoptotic effects in addition to its potent antioxidant actions. Therefore, melatonin may be useful in the treatment of tumors in association with chemotherapy drugs.

Objective: This study was performed to study the role of melatonin receptors on the cytotoxicity and apoptosis induced by the chemotherapeutic agents cisplatin and 5-fluorouracil in two tumor cell lines, such as human colorectal cancer HT-29 cells and cervical cancer HeLa cells.

Methods: MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was used to evaluate cytotoxicity of the different compounds. Caspase-3 activity was determined from the cleavage of its specific fluorogenic substrate (AC-DEVD-AMC). Substrate cleavage was measured with a microplate reader with excitation and emission wavelengths of 360 nm and 460 nm, respectively. Also, apoptotic cell death was analysed by redistribution of phosphatidylserine (PS) in the presence of PI.

Results: We found that both melatonin and the two chemotherapeutic agents tested induced a decrease in HT-29 and HeLa cell viability. Furthermore, melatonin significantly increased the cytotoxic effect of chemotherapeutic agents, particularly, in 5-fluorouracil-challenged cells. Stimulation of cells with either of the two chemotherapeutic agents in the presence of melatonin further increased caspase-3 activation. Concomitant treatments with melatonin and chemotherapeutic agents augmented the population of apoptotic cells compared to the treatments with chemotherapeutics alone. Blockade of MT1 and/or MT2 receptors with luzindole or 4-P-PDOT was unable to reverse the enhancing effects of melatonin on both cytotoxicity, caspase-3 activation and the amount of apoptotic cells evoked by the chemotherapeutic agents, whereas when MT3 receptors were blocked with prazosin, the synergistic effect of melatonin with chemotherapy on cytotoxicity and apoptosis was reversed.

Conclusion: Our findings provided evidence that in vitro melatonin strongly enhances chemotherapeutic-induced cytotoxicity and apoptosis in two tumor cell lines, namely HT-29 and HeLa cells and, this potentiating effect of melatonin is mediated by MT3 receptor stimulation.

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Melatonin, chemotherapy, cytotoxicity, apoptosis

P1-03

EF-HAND DOMAIN FAMILY MEMBER B REGULATES STORE-OPERATED CALCIUM ENTRY AND MIGRATION IN BREAST CANCER CELLS

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Precise regulation of Ca^{2+} signaling and homeostasis is essential for a number of cellular processes. Store-operated calcium entry (SOCE) is a major Ca^{2+} entry pathway in non-excitable cells but is also present in electrically excitable cells [1]. SOCE and its key mediators STIM, Orai and TRPC proteins are finely-tuned by a number of molecules and proteins, such as IP3 and calmodulin that orchestrate the whole mechanism. Alteration in SOCE, its main components or its regulators has been linked to a number of pathological conditions, including cancer [2]. STIM, Orai and TRPC proteins, as well as their regulators, are expressed in breast cancer cells with different levels of expression and function. By using molecular biology, biochemistry and fluorescence imaging microscopy techniques we have found that the EF-Hand domain family member B (EFHB), a novel regulator of SOCE, is highly expressed in the ER+ MCF7 and triple negative MDA-MB-231 breast cancer cell lines. Next, we have demonstrated that EFHB knockdown in both cell lines inhibits SOCE, which, subsequently, impairs proliferation, migration and invasion, which are relevant breast cancer features. Altogether, our results show an important role of EFHB fine-tuning SOCE and proliferation, migration and invasion in breast cancer cells.

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SOCE, TRP channels, EFHB, Breast Cancer

P1-04

TMEM97 IS INVOLVED IN THE REGULATION OF SOCE IN MDA-MB-231 CELLS

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TMEM97 (transmembrane protein 97) has recently been involved in the lipid metabolism and it is downregulated in the Newman-Pick disease [1]. Recent studies evidenced the presence of TMEM97 in breast cancer cells [2]; nonetheless, its function in these cells remains elusive. We confirmed by Western blotting the overexpression of TMEM97 in the triple negative breast cancer cells, MDA-MB-231, as compared to the non-tumoral cells, MCF10A. Using siRNA TMEM97 in MDA-MB-231 we found a reduction in the cells immunostaining with the specific anti-TMEM97 antibody, but not in the staining with NO1, the specific fluorescent ligand

of Sigma 2 receptor. Calcium imaging experiments using the GFP-TMEM97 overexpression plasmid and siRNA TMEM97, revealed a possible positive role of TMEM97 in thapsigargin-evoked calcium entry, but without affecting calcium release from the intracellular stores. Interestingly, in cells where the expression of TMEM97 was silenced using siRNA, incubation with the Sigma 2 receptor antagonist, SM21 (100 nM), still enhanced store-operated calcium entry (SOCE), so it does in WT cells. Immunoprecipitation experiments revealed a weak association of TMEM97 with the STIM1/Orai1 complex during SOCE activation in MDA-MB-231 cells. Altogether, our findings indicate that TMEM97 could be relevant for SOCE, nonetheless the exact mechanism remains unsolved.

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Calcium, TMEM97, Western Blotting

P1-05

STIM1 PHOSPHORYLATION AT Y316 EVOKES SARAF/STIM1 COMPLEX DISSOCIATION AND FACILITATES SOCE IN NG115-401L NEUROBLASTOMA CELLS

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Store Operated Calcium Entry (SOCE) is a major mechanism of calcium influx in non-excitabile cells. The endoplasmic reticulum calcium sensor, STIM1, undergoes structural and conformational changes in order to communicate to the ICRAC channel, Orai1 [1]. Recently, post-translational modification of STIM1 by phosphorylation has been postulated as a possible mechanism for facilitating STIM1/Orai1 interaction [2-3], which is aimed here by using the STIM1 Y316F mutant. In our hands, STIM1 Y316F overexpression in HEK293 cells, as well as in NG115-401L neuroblastoma cells (lacking STIM1 native expression), resulted in a reduction of STIM1 tyrosine phosphorylation evoked by thapsigargin (TG), and inhibition of calcium entry and ICRAC. Using the STIM1 fragments WT-OASF (233-476) and WT-OASF extended (233-525), and their respective Y316F mutants, we demonstrate that tyrosine phosphorylation did not participate in the conformational changes of the STIM1-OASF region, as demonstrated by FRET. On the other hand, SARAF has been recently described as a negative regulator of STIM1 [4]. TG-evoked STIM1 Y316 phosphorylation impairs STIM1/SARAF interaction in NG115-401L cells, thus facilitating SOCE in these cells. Immunoprecipitation and calcium imaging experiments in NG115-401L cells, overexpressing either STIM1 WT or STIM1 Y316F, and in the absence or presence of Sh SARAF, demonstrated that STIM1 Y316 phosphorylation evoked by TG impairs SARAF/STIM1 association, and facilitates its association with Orai1; then conducting bigger calcium entry.

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NG115-401L, SOCE, STIM1, Y316F, SARAF

P1-06

(-)-OLEOCANTHAL IMPAIRS PROLIFERATION AND MIGRATION IN TRIPLE NEGATIVE BREAST CANCER CELLS

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Triple negative breast cancer is the most severe and aggressive among breast cancer types. The absence of hormone receptors leads to negligible or in-existent respond to hormonal therapies, thus more drastic therapeutic strategies must be applied, leading to undesired side effects such as cytotoxicity on healthy tissues. Therefore, there is an obvious necessity for the characterization of new anti-tumoral compounds that attack the malignant cell while have no effect on non-tumoral cells. By using organic chemistry, molecular biology, biochemistry and fluorescence imaging microscopy techniques we have isolated a phenolic compound from olive oil, (-)-oleocanthol (OLCT), which selectivity inhibits cell proliferation in the triple negative MDA-MB-231 breast cancer cell line, having no effect over non-tumoral MCF10A cell proliferation. Furthermore, cell migration was impaired in the triple negative cell line with negligible effect in the non-tumoral one. Finally, we found that OLCT evoked Ca²⁺ entry in the triple negative MDA-MB-231 but not in the non-tumoral MCF10A breast cancer cell lines. OLCT-triggered Ca²⁺ mobilization was impaired by silencing TRPC6, which is over-expressed in MDA-MB-231 when compared with MCF10A. Altogether, our results show that OLCT has the ability to impair triple negative MDA-MB-231 proliferation and migration by triggering Ca²⁺ influx through the TRPC6 channels while having no effect in the non-tumoral MCF10A cell line.

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P1-07

GENETIC AND EPIGENETIC EVENTS IN THE REGULATION OF THE EXPRESSION OF THE ORGANIC CATION TRANSPORTER OCT1: ROLE IN CHEMORESISTANCE OF LIVER CANCER TO SORAFENIB

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Introduction: The multi tyrosine kinase inhibitor, sorafenib is used in the treatment of liver cancer although frequently patients present low response rates. One possible reason for the poor response to sorafenib is the impaired ability of cancer cells to transport it across the plasma membrane mainly via OCT1 (SLC22A1 gene), which is downregulated in primary liver cancer, both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA).

Aim: To study the events accounting for the downregulation of OCT1 in liver tumors and evaluate the interest of selective gene therapy strategies to overcome this limitation.

Methods: OCT1 mRNA expression was measured by RT-qPCR. Splicing isoforms of OCT1 were evaluated by PCR using specific primers. Data obtained from “The Cancer Genome Atlas” (TCGA) regarding HCC and CCA samples were used to evaluate the methylation levels of OCT1 promoter. In vitro and in vivo models were used to study the expression of OCT1 and the uptake of sorafenib that was measured by HPLC-MS/MS. Among miRNAs highly expressed in liver tumors (TCGA), six miR-candidates were identified from in silico analysis as possible targeting on OCT1 mRNA. These were cloned and tested in cell lines. Lentiviral and adenoviral vectors were developed to overexpress OCT1 and evaluate its effect on sorafenib response.

Results: Gene expression and DNA methylation of SLC22A1 were analyzed in HCC and CCA biopsies and TCGA data. Decreased OCT1 mRNA correlated with hypermethylation status of the SLC22A1 promoter. Treatment of HCC and CCA cells with decitabine (demethylating agent) or butyrate (histone deacetylase inhibitor), restored OCT1 expression and increased sorafenib uptake. High proportion of aberrant OCT1 mRNA splicing and up-regulation of hsa-miR-330 able to favor OCT1 mRNA degradation, may contribute to OCT1 decay in HCC and CCA. In contrast, lentiviral-mediated transduction of OCT1 in HCC and CCA cells enhanced sorafenib uptake and hence its cytostatic activity. HCC and CCA murine models also showed reduced Oct1 expression and impaired sorafenib uptake. In an orthotopic model of CCA in immunodeficient mice, intrahepatic tumors were chemoresistant to sorafenib. However, tumor growth was markedly reduced by the co-treatment of animals with sorafenib and adenoviral vectors encoding OCT1 under the control of a tumor selective promoter activated in CCA.

Conclusions: Several epigenetic events are involved in OCT1 decay in HCC and CCA tumors. Gene therapy able to selectively induce OCT1 in tumor cells, but not in adjacent healthy liver tissue, could be a useful chemosensitizing strategy to improve their response to sorafenib.

OCT1, Transport, Cancer, Chemotherapy, Liver

P1-08

SIGMA-2 RECEPTOR IS A NEGATIVE REGULATOR OF STORE-OPERATED Ca^{2+} ENTRY IN MDA-MB-231 CELLS

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Store-operated Ca^{2+} entry (SOCE) is one of the most relevant mechanisms for the regulation of intracellular Ca^{2+} homeostasis in non-excitabile cells. SOCE regulates relevant steps of the cell cycle [1] and is involved in the development of apoptosis [2]. Sigma-2 receptor ($\sigma 2R$) is overexpressed in certain cancer cells [3]. Our results indicate that treatment of the triple negative breast cancer cell line MDA-MB-231 with the $\sigma 2R$ -antagonist (SM-21; 100nM) enhanced SOCE evoked by thapsigargin (TG). In contrast, SOCE was not affected by SM-21 in the non-tumoral breast cell line MCF-10A. Treatment of MDA-MB-231 cells with TG enhances $\sigma 2R$ /STIM1 interaction, which was impaired by cell pre-treatment with SM-21. Finally, preincubation of MDA-MB-231 cells with SM-21 facilitated the STIM1/Orai1 coupling by itself, without having any significant effect on TG-evoked STIM1/Orai1 interaction in MDA-MB-231 cells. Altogether, we provide a mechanistic explanation for $\sigma 2R$ function in MDA-MB-231 breast cancer cells, through the regulation of SOCE.

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Sigma-2 receptor, SOCE, STIM1, Orai1

P1-09

PROTECTIVE EFFECT OF MELATONIN ON CELL MEMBRANE FLUIDITY IN A HEPATIC ISCHEMIA-REPERFUSION MODEL

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Introduction: During hepatic ischemia (I) ATP cellular levels decrease. On the other hand, during reperfusion (R), abundant reactive oxygen species (ROS) are generated, which initiate lipid peroxidation (LP) in cell membranes. Melatonin (MEL) is a potent antioxidant derived from tryptophan and secreted in the pineal gland as well as many other organs. Its antioxidant activity is due to the capacity to neutralize free radicals by electronic cession and its ability to stimulate antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase. **Objective:** To analyze the effects of ischemia-reperfusion injury (I+R) and treatment with MEL on the cell membranes fluidity isolated from rat liver tissue.

Material and methods: 37 male Sprague-Dawley rats (225-250 g) were randomly distributed into four groups: Control (n=8), I (n=10), I+R (n=9), and MEL (50 mg/kg, ip) after I+R (n=10). All the experiments were realized during 50 minutes (20 minutes of ischemia and 30 minutes of reperfusion). MEL was provided 30 minutes before the initiation of the ischemia. Cell membrane fluidity was determined by fluorescence spectroscopy using TMA-DPH as a marker.

Results and discussion: Attending to cell membrane fluidity levels in control animals (3.791±0.031), no differences were observed after 20' of ischemia (3.837±0.058), although there was a decrease (p<0.0001) in fluidity after R (3.234±0.081). MEL (3,827±0.045) prevented membrane rigidity caused by R without statistically significant differences compared to the control group. These results agree with previous studies that observed that LP in cell membranes produces rigidity by 2 mechanisms: a) a reduction in the ratio of polyunsaturated/saturated fatty acids; and b) the formation of cross-lipid bonds. In addition, I+R initiate a cascade of phenomena caused by oxidative stress, inflammatory mediators, leukocyte recruitment and via formation of reactive nitrogen-dependent species.

Conclusions: Our results demonstrate that melatonin efficiently preserves cell membrane fluidity against hepatic I+R injury due to its free radical scavenging activity as well as its protective effect in cell membrane against LP.

Melatonin, oxidative stress, membrane-fluidity, liver, ischemia-reperfusion

P1-10

MELATONIN REDUCES LIPID PEROXIDATION IN HEPATIC HOMOGENATES IN AN ISCHEMIA-REPERFUSION MODEL

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Introduction: Lipid peroxidation (LP) is a self-degenerative chain reaction that destroys lipids of the cell membrane and contributes to the formation of cellular edema secondary to ischemia-reperfusion (I+R) injury. This produces an increase in the intracellular levels of Ca^{2+} and Na^+ , as well as a release of cytochrome C in the cytoplasm, with the subsequent activation of the caspases, which leads to cell lysis. Malondialdehyde (MDA) and the 4-hydroxyalkenals (4-HDA) are reactive oxygen species (ROS) generated during oxidative stress damage in the lipids of a tissue. Melatonin (MEL) is a powerful antioxidant and free radical scavenger that stimulates the activity of superoxide dismutase, glutathione peroxidase, and glutathione reductase, in addition to its ability to stimulate the immune system.

Objective: 1) To study lipid peroxidation measuring MDA and 4-HDA concentrations in an in vivo model of I+R in liver tissue homogenate. 2) Assess the utility of melatonin (MEL) by reducing the oxidative stress injury in that model. **Material and methods:** 37 male Sprague-Dawley rats (225–250 g) were randomly distributed into four groups: Control (n=8), ischemia (n=10), I+R (n=9), and MEL (50 mg/kg, intraperitoneally) after I+R (n = 10). All the experiments were realized during 50 minutes (20 minutes of ischemia and 30 minutes of reperfusion). MEL was provided 30 minutes before the initiation of the ischemia. MDA and 4-HDA concentrations were measured by spectrophotometry at 586 nm. **Results and discussion:** MDA and 4-HDA levels were similar in the control group (0.355 ± 0.041) and ischemia (0.334 ± 0.027). However, an increase of ROS secondary to LP generated during I+R damage (0.730 ± 0.063) was observed ($p < 0.001$) compared to the control group. MEL (0.359 ± 0.020) prevented these disturbs without statistically significant differences with control animals. Ischemia-reperfusion injury activates a cascade of oxidative stress injury due to the increase of inflammatory mediators, the recruitment of leukocytes and the increase of reactive nitrogen species. **Conclusions:** Oxidative stress is increased significantly during reperfusion. The administration of MEL prevented the formation of ROS during reperfusion due to its capacity to neutralize free radicals, which allows to the indoleamine to preserve cellular activity in situations of vascular I+R.

Melatonin, oxidative-stress, lipid-peroxidation, malondialdehyde, liver, ischemia-reperfusion

P1-11

LPA/LPA1 SIGNALLING IN MOTONEURON DEGENERATION: THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Changes in neuronal excitability are risk factors for neuronal death which occurs in a number of neurodegenerative diseases. Thus, uncovering molecular mechanisms that regulate neuronal excitability has both basic and clinical relevance. Membrane-derived phospholipids have emerged as intercellular messengers, however, little is known about their involvement in neurodegeneration. In this context, unpublished results from our lab have demonstrated that one of them, the lysophosphatidic acid (LPA), mainly acting through the isoreceptor LPA1, modulates motoneuron (MN) intrinsic membrane excitability, via ROCK-mediated regulation of the leak K^+ channel TASK1. Specifically, LPA induced a robust increase in MN excitability via LPA1. Thus, we hypothesize here on the toxicity for MNs of LPA/LPA1 signalling and/or its involvement in determining MN vulnerability to degeneration. To assess feasible toxicity on MNs of this lysophospholipid, we first studied the effect on the survival of primary cultures of spinal MNs of diverse concentrations (ranging from physiological to pathological concentrations) of LPA. In this way, lower LPA concentrations tested (up to 10 nM) did not affect SMNs survival. However, higher concentrations (from 100 nM to 100 mM) significantly reduced SMNs survival in a concentration-dependent manner. On the other hand, pre-treatment of SMNs with a small interfering RNA directed against mRNA for LPA1 (siRNA_{LPA1}) drastically reduced toxicity of LPA on SMNs as compared with cultures pre-treated with a non-targeting siRNA (cRNA). Interestingly, LPA1 overexpression was observed in both, SMNs obtained from embryos of the SOD1G93A mouse model of ALS (SMNsG93A), and the spinal cord of SOD1G93A mice at the pre-symptomatic stage. Furthermore, LPA resulted more toxic for SMNsG93A than for non-transgenic SMNs. Finally, we assess feasible neuroprotective effects of siRNA_{LPA1} on the SOD1G93A model of ALS. Weekly intracerebroventricular injection of siRNA_{LPA1}, beginning at a pre-symptomatic stage (2-month-old), delayed both, motoneuron loss in the lumbar spinal cord, and symptoms onset in SOD1G93A mice. siRNA_{LPA1} treatment also alleviated weight loss, improved motor performance (rotarod, footprint, runtime and grip strength), and prolonged survival time of SOD1G93A mice, as compared with cRNA-treated animals. Altogether, these outcomes indicate that a gain in LPA/LPA1 signalling is toxic for MNs. Preclinical findings support that LPA/LPA1 are potential therapeutic targets and, maybe biomarkers, for ALS.

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Neurodegeneration, motoneuron, ALS, LPA, SOD1G93A

P1-12

PROTECTIVE EFFECT OF MELATONIN ON CELL MEMBRANE FLUIDITY IN A RENAL ISCHEMIA-REPERFUSION IN VIVO MODEL

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Introduction: Melatonin, an indolamide derived from the essential amino acid tryptophan, has a powerful antioxidant activity against free radicals. Melatonin improves the immune system and protects against mitochondrial dysfunction caused by different toxins. During the process of

ischemia-reperfusion (IR), lipid peroxidation (LP) due to the overproduction of reactive oxygen species (ROS) occurs. **Objective:** 1) To analyze the effects of IR injury on fluidity of cell membranes isolated from rat renal tissue. 2) To assess the utility of melatonin as a protective agent in renal cell membranes preservation during the oxidative stress produced on this in vivo model.

Material and methods: 37 male Sprague-Dawley rats (225–250 g) were randomly distributed into four groups: 1. Control (n = 9); 2. Ischemia (n = 10); 3. Ischemia-Reperfusion (n = 8); 4. Melatonin + Ischemia-Reperfusion (n = 9). Ischemia time was 45 minutes. Reperfusion period was extended for 60 minutes. The dose of melatonin (50 mg / kg) was administered intraperitoneally 30 minutes before the onset of ischemia. Cell membrane fluidity was determined by fluorescence spectroscopy using TMA-DPH as a marker.

Results and discussion: There were no significant variations in cell membrane fluidity after 45 minutes of ischemia (3.524 ± 0.033) compared to the control group (3.512 ± 0.030), although membrane rigidity was observed after reperfusion (3.195 ± 0.027) ($p < 0.0001$). Melatonin prevented the decrease in cell membrane fluidity (3.496 ± 0.036) without statistically significant differences compared to the control group. Oxidative stress increases cell membrane rigidity through two mechanisms: a) reduction in ratio of polyunsaturated / saturated fatty acids in the membrane composition; b) formation of cross junctions of the lipid remains of the membrane, which limits its movement.

Conclusions: IR damage involves a cascade of oxidative stress that results in cell membrane rigidity secondary to LP process. Our results demonstrate that melatonin is a useful agent in the preservation of cell membrane stability against rigidity induced by increased oxidative stress during renal reperfusion.

Melatonin, membrane fluidity, kidney, ischemia-reperfusion.

P1-13

PROTECTIVE EFFECT OF MELATONIN ON MITOCHONDRIAL MEMBRANE FLUIDITY. ISCHEMIA-REPERFUSION HEPATIC MODEL

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Introduction: Ischemia-Reperfusion (IR) injury occurs when blood supply to a certain tissue is interrupted for a variable time (ischemia), to be subsequently restored (reperfusion). Mitochondrial dysfunction has been pointed out as a crucial circumstance in IR damage, so the protection of the mitochondria during this process can reduce its impact on multiple organs. Melatonin (MEL) is a powerful antioxidant derived from tryptophan that has been shown to mitigate mitochondrial dysfunction that occurs during IR processes. It is its protective effect on the mitochondria that makes MEL one of the most promising options for the treatment of IR injury.

Objective: 1) To analyze the effect of IR injury on the fluidity of mitochondrial membranes isolated from rat liver tissue. 2) To assess the utility of MEL as protective element of the mitochondrial membranes stability during the oxidative stress produced in an in vivo model of hepatic ischemia-reperfusion.

Material and methods: 37 male Sprague-Dawley rats (225–250 g) were randomly distributed into four groups: Control (n=8), I (n=10), I+R (n=9), and MEL (50 mg/kg, ip) after I+R (n=10). All the experiments were realized during 50 minutes (20 minutes of ischemia and 30 minutes of reperfusion). MEL was provided 30 minutes before the initiation of the ischemia. Mitochondrial membrane fluidity was determined by fluorescence spectroscopy using TMA-DPH as a marker.

Results and discussion: Mitochondrial membrane fluidity levels in control group (4.094 ± 0.043) did not present significant differences with respect to those obtained after 20 'of ischemia (3.994 ± 0.031), although there was a

fluidity decrease after reperfusion (3.631 ± 0.028).) ($p < 0.0001$). MEL (4.038 ± 0.030) prevented membrane rigidity caused by R without statistically significant differences compared to the control group.

Conclusions: Attending our results, MEL is a useful agent in preservation of mitochondrial membrane stability against hepatic IR, thus contributing to mitigate IR damage.

Melatonin, mitochondrial dysfunction, liver, ischemia-reperfusion

P1-14

ROLE OF ORAI1 AND SARAF IN VASCULAR SMOOTH MUSCLE CELLS PROLIFERATION

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Store-Operated Calcium Entry (SOCE), a mechanism for Ca^{2+} influx regulated by intracellular Ca^{2+} stores, plays an essential role in several cellular functions. It is known that the key molecular components of Store-Operated Ca^{2+} Channels (SOCC) are Orai1, SOCC subunit, and Stromal Interaction Molecule 1 (STIM1), endoplasmic reticulum Ca^{2+} sensor. However, details of how these channels are regulated in vascular cell proliferation still remain uncertain. Here, we focused our investigation in examining the role of SARAF (SOCE Associated Regulatory Factor), a new regulatory protein involving in SOCE in proliferative Vascular Smooth Muscle Cells (VSMC).

Objective: The aim of this study was to examine the role of SARAF in VSMC proliferation and neointima formation after balloon injury of rat carotid arteries.

Materials and Methods: Experiments were conducted in an animal model of rat carotid angioplasty, one, two or three weeks after carotid injury, to study the vascular wall thickening. We also employed VSMC isolated from rat coronary artery to assay intracellular Ca^{2+} mobilization and cell proliferation.

Results: We confirm the formation of neointima after balloon injury of rat carotid arteries by haematoxylin and eosin staining of tissue sections. Next, we found significant higher expression of SARAF in injured arteries compared to control tissues, corroborating the presence of this regulatory protein in the neointima layer. We also observed that SARAF expression markedly colocalize with Orai1 in this area. Furthermore, we found that selective silencing of SARAF gene by small interfering RNA (siRNA) evoked an increase of $[Ca^{2+}]_i$ in VSMC when were treated with Insulin-like Growth Factor-1 (IGF-1), meanwhile silencing of Orai1 inhibited IGF-1 induced $[Ca^{2+}]_i$ increase.

Conclusions: Our data suggest that SARAF modulate Ca^{2+} mobilisation in presence of a vasoactive agonist, IGF-1, which could regulate SOCE and VSMC proliferation after the vessel injury produced as a result of an angioplasty.

SARAF; Orai1; Smooth muscle proliferation

P1-15

EFFECT OF CEREBROLYSIN ON THE AMPLITUDE OF NMDA, AMPA AND GABA CURRENTS RECEPTOR MEDIATED AT THE CA1 SYNAPSIS REGION OF THE HIPPOCAMPUS

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Cerebrolysin (CBL) is a preparation produced by enzymatic decomposition of purified brain proteins from the pig. CBL has been used in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. The action mechanisms of this mix of compounds are not well understood. The aim of the present work was to difference the effect of CBL on AMPA, NMDA and GABA currents in the CA1 region of the hippocampus. The hippocampus is a structure directly related to memory and learning processes, navigation and map formation, playing a important role in the cognitive decline associated to some neurodegenerative diseases. The experiments were carried out in the CA1 region of hippocampal slices obtained from the brain of P13-P90 of C57BL/6 mice using the whole-cell configuration of the Patch-Clamp technique. The results showed that: 1) CBL decreased the amplitude of NMDA receptor-mediated EPSCs (18 ± 6 , $n=5$), AMPA receptor-mediated EPSCs (11 ± 7 , $n=5$) and GABA_A receptor-mediated IPSCs (53 ± 7 , $n=8$). In conclusion, Cerebrolysin deprimates excitatory and inhibitory synaptic transmission in the CA1 region of the hippocampus.

Cerebrolysin, currents, hippocampus, CA1

P1-16

CEREBELLAR KAINATE RECEPTOR-MEDIATED FACILITATION OF GLUTAMATE RELEASE REQUIRES Ca^{2+} -CALMODULIN AND PKA

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We elucidated the mechanisms underlying the kainate receptor (KAR)-mediated facilitatory modulation of synaptic transmission in the cerebellum. In cerebellar slices, KA ($3 \mu\text{M}$) increased the amplitude ($138 \pm 11\%$) of evoked excitatory postsynaptic currents (eEPSCs) at synapses between axon terminals of parallel fibers (PF) and Purkinje neurons. KA-mediated facilitation was antagonized by NBQX ($95 \pm 4\%$) under condition where AMPA receptors were previously antagonized. Inhibition of protein kinase A (PKA) suppressed the effect of KA on glutamate release ($88 \pm 3\%$), which was also obviated by the prior stimulation of adenylyl cyclase (AC). KAR-mediated facilitation of synaptic transmission was prevented by blocking Ca^{2+} permeant KARs using philanthotoxin ($75 \pm 5\%$). Furthermore, depletion of intracellular Ca^{2+} stores by thapsigargin ($77 \pm 5\%$), or inhibition of Ca^{2+} -induced Ca^{2+} -release by ryanodine ($82 \pm 4\%$), abrogated the synaptic facilitation by KA. Thus, the KA-mediated modulation was conditional on extracellular Ca^{2+} entry through Ca^{2+} -permeable KARs, as well as and mobilization of Ca^{2+} from intracellular stores. Finally, KAR-mediated facilitation was sensitive to calmodulin inhibitors, W-7 and calmidazolium ($86 \pm 3\%$ and $78 \pm 11\%$, respectively), indicating that the increased cytosolic $[Ca^{2+}]$ sustaining KAR-mediated facilitation of synaptic transmission operates through a downstream Ca^{2+} /calmodulin coupling. We conclude that, at cerebellar parallel fiber-Purkinje cell synapses, presynaptic KARs mediate glutamate release facilitation, and thereby enhance synaptic transmission through Ca^{2+} -calmodulin dependent activation of adenylyl cyclase/cAMP/protein kinase A signaling.

Kainate receptors, glutamate, presynaptic, Ca^{2+} -calmodulin, PKA

P1-17

ILLUMINATING TRANSCRIPTOMICS OF CALCIUM REMODELING IN COLORECTAL CANCER

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Colorectal cancer (CRC) cells undergo a remodeling of intracellular Ca^{2+} homeostasis that contributes to cancer hallmarks such as enhanced proliferation, invasion and survival. Important changes at the functional level include upregulation of store-operated Ca^{2+} entry (SOCE) and mitochondrial Ca^{2+} uptake together with depletion of intracellular Ca^{2+} stores (Sobradillo et al., J Biol Chem 2014; Hernández-Morales et al., Oncotarget 2017). Some of these changes have been investigated at the molecular level. However, since nearly 250 genes are involved in intracellular Ca^{2+} transport we have assessed possible differences in expression of these genes using Next Generation Sequencing (NGS) technologies. For this end, we used normal human colonic NCM460 cells and human colon adenocarcinoma HT29 cells. We had previously used IonTorrent® technology for analysis of differential expression of 77 genes (Pérez Riesgo et al., Int J Mol Sci 2017). Now, we have extended our analysis to all calcium related genes and the whole transcriptome of NCM460 and HT29 cells using Illumina® technology. Data analysis has been carried out using R and bioconductor packages. We found that the outcomes from the 77 genes reported previously were very similar using both IonTorrent® and Illumina® technologies. Specifically, we observed similar differences in expression of selected voltage-operated Ca^{2+} channels and molecular players involved in store-operated Ca^{2+} entry (SOCE). Moreover, changes in expression of TRP channels, Ca^{2+} release channels, Ca^{2+} pumps and Na^+/Ca^{2+} exchangers were similar in both cases. Finally, genes involved in activation of mitochondria Ca^{2+} transport were similarly upregulated in CRC while genes involved in preventing mitochondrial Ca^{2+} uptake were downregulated. These results may provide a comprehensive view of calcium remodeling in CRC and its contribution to cancer hallmarks.

Calcium, colon cancer, omic data analysis

P1-18

EFFECT OF CEREBROLYSIN IN THE CA1 REGION OF THE HIPPOCAMPUS

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Cerebrolysin (CBL) is a preparation produced by enzymatic decomposition of purified brain proteins from the pig. CBL has been used in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. However, the action mechanisms of this mix of compounds is still unknown. The hippocampus is a structure that has been directly involved in learning and memory processes, navigation and map formation. The hippocampus seems to play a fundamental role in the cognitive decline present at same neurodegenerative diseases. The aim of the present work was to study the effect of Cerebrolysin in the CA1 region of the hippocampus. Experiments in the CA1 region of hippocampal slices prepared from P30-P90 mice, using extracellular recordings (field recordings) and intracellular recordings (whole-cell configuration of the patch-clamp technique). The

results showed that: CBL reversibly decreased EPSP slope in extracellular ($44 \pm 15 \%$) and intracellular ($13 \pm 2 \%$) recordings, and CBL depolarizes membrane potential in the pyramidal neurons of the region CA1 in the hippocampus (Baseline: $77 \pm 3 \%$; CBL: $46 \pm 5 \%$). In conclusion, Cerebrolysin depresses excitatory synaptic transmission at the synapses established between the Schaffer Collaterals and CA1 pyramidal neurons. In addition, CBL despolarizes CA1 pyramidal neurons.

Cerebrolysin, hippocampus, CA1, recordings

P1-19

THE AUTOTAXIN INHIBITOR PF-8380 ALTERS INTRINSIC MEMBRANE PROPERTIES OF HYPOGLOSSAL MOTONEURONS IN VITRO

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Autotaxin (ATX) is a lysophospholipase D able to hydrolyze lysophosphatidyl choline to lysophosphatidic acid (LPA). We recently observed that LPA modulates intrinsic membrane excitability of hypoglossal motoneurons (HMNs), which, in turn, could influence neuronal plasticity and vulnerability to excitotoxic death. Interestingly, ATX has been proposed as a new target for cortical hyperexcitability in psychiatric disorders by modulating excitatory/inhibitory balance (Mol Psychiatry. 2018. doi: 10.1038/s41380-018-0053-1). However, ATX could mediate excitability alterations of neuronal networks by directly modulating intrinsic membrane properties of neurons. In this way, we studied whether baseline activity of ATX regulates resting membrane potential and input resistance of HMNs in vitro by incubating brainstem slices for 10 min with the ATX inhibitor PF-8380 (1 μM). Whole cell patch-clamp recordings reported that PF-8380 induced membrane potential hyperpolarization ($-10.4 \pm 2.3 \text{ mV}$; $p < 0.001$, paired t-test) in 14 of 16 and reduced input resistance ($-28.4 \pm 6.0 \text{ MOhms}$; $p < 0.001$) in 13 of 16 recorded HMNs. These outcomes indicate that endogenous activity of ATX in our experimental conditions controls passive membrane properties of HMNs. Alterations in membrane excitability induced by the ATX inhibitor agree with the previous results obtained after addition of the ATX product LPA. Since LPA induced membrane potential depolarization and increased input resistance of HMNs, it can be argued that the ATX-LPA axis regulates neuron excitability and point to deregulation of this signaling pathway as a feasible mechanism involved in neuronal excitability disruption and vulnerability associated to multiple neurological conditions.

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Motoneuron, excitability, ATX, LPA

P1-20

LIPID RAFT IMPAIRMENT IN NEURONAL MEMBRANES CORRELATES WITH ALPHA-SYN OLIGOMERIZATION IN A MOUSE MODEL OF PARKINSON DISEASE

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Parkinson Disease (PD) is a neurodegenerative disease associated with ageing that mainly affects the substantia nigra pars compacta and other brain areas. Selective death of dopaminergic neurons is associated with the formation of Lewy bodies. Alpha synuclein is the main component of these Lewy bodies, and hence it has been identified as a key protein for the development of PD. This protein requires the interaction with specific lipid classes to promote toxic self-aggregation. In previous work, our group has found profound alterations in the distribution of structural lipids in the cortical Lipid Rafts (LR) from PD patients. The aim of this study is to identify alterations in gangliosides and alpha-Syn distribution in lipid raft fractions that might be involved in the development of PD. Here we have used the murine model of PD treated with MPTP as neurotoxic. Four different age- and MPTP-treated experimental groups (W6, control 6-months old mice; M6, MPTP-treated 6-months old mice; W14, control 14-months old mice; and M14; MPTP-treated 14-months old mice) were established. Lipid Rafts from different brain areas, including cortex and cerebellum were isolated in order to study the distribution of alpha-Syn and gangliosides among Raft (LR) and non-Raft (NR) membranes. Monomeric and aggregated alpha-Syn was detected by western blot using specific antibodies. The brain major ganglioside classes were analyzed by slot blot using Cholera toxin coupled to HRP for GM1 detection and specific antibodies against GD1a, GD1b and GT1b. Alpha-Syn was widely found in the NR fraction both in the cortex and the cerebellum. In the cortical area, -Syn oligomers were found as a result of, both, aging and MPTP treatment. In contrast, the ratio aggregated alpha-Syn / total alpha-Syn remained unchanged in the cerebellum. Furthermore, the amount of phosphorylated-Syn forms was increased in the insoluble oligomers. In correlation with this, ganglioside distribution in LR was reduced during ageing and MPTP-induced neurotoxicity. These data demonstrate a correlation of LR-related ganglioside detriment with toxic alpha-Syn aggregates as a parameter of pathological PD progression.

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Parkinson disease, alpha synuclein, lipid rafts

1-21

BASICS OF THE PATCH-CLAMP TECHNIQUE

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Patch-clamp is an electrophysiological technique used in the study of both active and passive electrical properties of cells. It is based on the generation of a high resistance seal between the recording electrode and the cell membrane which makes the recording of currents through ion channels expressed in the cell membrane possible. This has allowed us to characterize the channels expressed in different cell types and to further study their biophysical characteristics. The aim of this project is to learn the basis of the patch-clamp technique and to apply them to record potassium currents in the isolated vascular smooth muscle cells (VSMC). We have

carried out a bibliographic review in PubMed (descriptors: “patch clamp technique”, “isolated vascular smooth cells”) and in the department’s bibliographical files. In addition, we have isolated VSMCs of rat mesenteric artery and recorded ion currents expressed in them. This work presents the theoretical principles of the Patch-Clamp technique, in the “whole cell” configuration and summarizes the process carried out in isolated VSMC to record cell currents. Furthermore, we have recorded voltage-dependent outward K^+ currents in the VSMCs, which regulate the relaxation of the vascular smooth muscle in the rat mesenteric artery.

Patch-clamp, potassium channels

P1-22

HEART RATE VARIATION IN HORSES AS ADAPTATION RESPONSE TO WATER TREADMILL

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Water treadmill (WT) is a treadmill submerged in water that allows the regulation on the water level and the speed. It is a rehabilitation and training method that reduces the pressure on muscle, joints and tendons. The objective of this research was to observe the horses’ adaptation to WT in continuing sessions, using the measure of their heart rate (HR). Five horses of different breeds from the Equine Sport Medicine Center (CEMEDE), belonging to the Faculty of Veterinary, University of Cordoba, performed 4 sessions of acclimation of between 15 and 30 minutes of duration. The sessions were analyzed in 5 events: (1) first up and down of the WT, (2) second up and down of the WT, (3) noise produce by the WT, (4) exercise without water and (5) exercise with water. The exercise was at 6 Km/h. The results of the sessions 0 to 3 were: in the event (1): 92.75±16.83; 101.6±13.27; 98.75±17.11 and 111.6±21.77 b.p.m. In the event (2), 80.33±21.57; 91±13.58; 78.75±17.87 y 97.4±23.54 b.p.m. In the event (3), 92.25±21.79; 76.20±11.32; 56.5±6.02 y 76±16.67 b.p.m. In the event (4), 98.66±21; 91.2±16.81; 74.52±19.34 y 86±8.6 b.p.m. In the event (5) there was not session 0, the data from session 1 to 3 are presented, 96±7.31; 77.25±10.87 y 93.8±10.56 b.p.m. There was no statistical difference between any of the measures within events ($p>0.05$). In conclusion, there is a tendency to increase the HR in the event (1) at each session. In the event 2 the HR tend to stay steady during the sessions. However, in the events 3, 4 and 5, a decrease on HR form sessions 0 to 2 and an increase in session 3 was observed, this may be due to a longer period of time between sessions 2 and 3. The protocol used in this research could be improved for future researches.

Acclimation; heart rate; horse; water treadmill

P1-23

THE PILATES THERAPEUTIC METHOD IMPROVES MOOD, WELL-BEING AND QUALITY OF SLEEP IN PATIENTS WITH CHRONIC SPINAL PAIN

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Chronic spinal pain is one of the main causes of disability and loss of quality of life. The aim of the present study was to determine the effect of a 3-month therapeutic Pilates program on mood, well-being and subjective quality of sleep in patients with chronic spinal pain. **Material and methods:** 10 patients with chronic spinal pain took part in the study. A series of basic and intermediate therapeutic Pilates exercises were performed in the physiotherapy clinic CEKINESIA S.L. Sessions were held for 3 months, two alternate days per week, 60 minutes per session. To evaluate the effect of the intervention, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), WHO5 Well-Being Index and the Pittsburgh Sleep Quality Index (PSQI) were used. Patients completed the questionnaires before and after three months of intervention.

Results: The levels of depression, evaluated through the BDI, and the subjective quality of sleep, through the PSQI, significantly decreased their scores. Similarly, there was a significant increase in the scores obtained in the WHO5 Well-Being Index. However anxiety levels only showed an improvement trend.

Conclusions: The results obtained suggest that the performance of therapeutic Pilates improves mood status, the subjective quality of sleep and general well-being in patients with chronic spinal pain.

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Pilates therapeutic, mood, chronic spinal pain

P1-24

QUALITY OF SLEEP AND ANXIETY IN DIFFERENT SPORTING CATEGORIES

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Introduction: The human being has the physiological need to sleep to recover the energy lost in the performance of daily activities, and thus be able to maintain health. The quality of sleep is, therefore, a clinical aspect of enormous relevance and this is shown by the statistics in this regard that confirm that 30–40% of the population suffers from insomnia. In the case of athletes, sleep has been considered a key factor for competition and their performance, being the most common alterations among athletes partial sleep deprivation and fragmented sleep.

Objective: Our objective was to analyze the relationship between sleep quality and precompetitive anxiety, in different sports categories according to sex.

Materials and methods: A total of 83 athletes (42 men and 41 women) without physical or psychological pathologies, participated voluntarily in the study. All of them belonged to federated sports teams of the Extremadura region. The data were obtained in individuals between the ages of 18 and 33, with an average of $c=23,37 \pm 3,85$ years. All data were obtained under the approval of the Bioethics Committee. Participants completed the Pittsburgh Sleep Quality Test (PSQI) and the State-Trait Anxiety Inventory (STAI). The latter includes separate scales of self-assessment that measure two independent concepts of anxiety: Anxiety-State (AS) and Anxiety Trait (AT). In this study, only the Anxiety-Trait scale was used.

Results: Our results show that women have worse values in the sleep test than men, regardless of the type of sport performed. In

both sexes, athletes presented higher levels of anxiety than the control group, with female athletes having higher levels of anxiety than male athletes. Discussion: The worse quality of sleep of the women with respect to the men in the sports categories can be due to the greater anxiety that they maintain. In addition, this anxiety is greater in sports such as Crossfit and Basketball.

Conclusion: We can conclude that there is a correlation between the highest score in anxiety levels and poor sleep quality. **Acknowledgments:** This research has been supported by the Junta de Extremadura - Fondo Europeo de Desarrollo Regional (FEDER) GR18040.

Sleep, anxiety, sports categories

P1-25

INFLUENCE OF A CONCURRENT EXERCISE TRAINING PROGRAM DURING PREGNANCY ON THE PLACENTA MITOCHONDRIAL DNA INTEGRITY AND THE LEVELS OF MINERALS WITH ENZYMATIC RELEVANCE. THE GESTAFIT PROJECT

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Exercise has shown positive effects during pregnancy on several maternofetal metabolic markers. Despite that, information about exercise on placental physiology is scarce. Placenta is imperative because it provides foetal nutrient uptake, thermo-regulation, waste elimination, immunoprotection, hormonal production and gas exchange. Very related to placental function is the integrity of mitochondria and mitochondrial DNA (mtDNA). Thus, alterations in mtDNA density (copy number) have been related to a higher risk of placental abruption. Moreover, mitochondrial mutations such as deletions, may be important as placenta maturation and aging happen. This is because such alterations can condition bioenergetics capacity of this tissue and its exposure to oxidative stress. Certain minerals are involved in mitochondria and cell integrity and function. Manganese is present in the antioxidant enzyme mitochondrial superoxide dismutase (SOD). Iron is needed for heme but also for iron sulfur cluster-containing proteins at the electron transport chain and for oxidative phosphorylation or DNA synthesis. Selenium is present in the enzymes glutathione peroxidase and thioredoxin reductase. Zinc is necessary for DNA synthesis and cytoplasmic SOD. Magnesium stabilizes DNA, RNA and is important in the activity of enzymes and ATP related reactions.

Study aims: i) To investigate the influence of an exercise intervention during pregnancy on the placental mtDNA copy number and complex I deletion and, ii) To explore the association of these mitochondrial markers with the concentration of minerals with antioxidant coenzymatic relevance.

Methodology: Forty-six pregnant women were pseudo-randomized into exercise or control group. Exercise group followed a concurrent

aerobic and strength training program, three 60-min sessions/week, from the 17th gestational week until delivery. Placenta was extracted and processed for minerals determination by inductively coupled plasma mass spectrometry technique. RT-PCR was used to determine mtDNA copy number and ND1/ND4 deletion. **Results:** Mothers performing exercise presented greater mitochondrial copy number and lower mitochondrial DNA deletion. Placenta from mothers performing exercise presented higher concentrations of manganese than those from the control (0.26 ± 0.03 mg/dL vs. 0.13 ± 0.03 mg/dL). Manganese content was associated with lower mitochondrial DNA deletion ($r = -0.382$) and greater copy number ($r = 0.513$). Iron content was associated with higher copy number ($r = 0.393$). Selenium was associated with lower mtDNA deletion ($r = -0.377$) and greater copy number ($r = 0.442$). Zinc and magnesium were associated with higher copy number ($r = 0.447$ and $r = 0.453$, respectively).

Conclusion: This concurrent exercise training program induced a better placental status, which might be mediated through an improvement of mitochondrial bioenergetics and antioxidative capacity.

Exercise training, placenta, mitochondria, minerals, DNA

P1-26

INFLUENCE OF OBESITY AND HABITUAL EXERCISE ON THE β 2 ADRENERGIC REGULATION OF PHAGOCYTTIC AND MICROBICIDE CAPACITY OF MACROPHAGES FROM C57BL/6J MICE

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Introduction: Obesity is a worldwide epidemic and it is associated with comorbid conditions that involve alterations of the innate immune response. It is also correlated with changes in the function of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. Catecholamines secreted by the sympathetic nervous system and the adrenal glands are important immunoregulatory molecules, and adrenergic agonists interfere with the inflammatory response. However, the influence of obesity on the adrenergic regulation of the immune system (particularly the innate/inflammatory response), and the influence of regular exercise on the mechanisms underlying this regulation in obesity, has been not studied yet. **Objective:** The aim was to determine the influence of obesity, and a program of habitual exercise in this condition, on the β 2-adrenergic regulation of the phagocytic and microbicide capacity of peritoneal macrophages from C57BL/6J mice. **Methods:** A group of 24 eight-week old C57BL/6J mice were fed a diet containing either 5% (lean group, $n = 12$) or 40% of fat (obese group, $n = 12$) for 18 weeks. After 10 weeks, 6 animals from each group were subjected to a habitual exercise program (running, 3 days/week for 45 min at 18 m/min for 8 weeks). The phagocytic and microbicide capacity of macrophages against opsonized bacteria was evaluated by flow cytometry in peritoneal macrophages, in the presence or absence of the β 2 adrenergic agonist terbutaline. β 2-adrenergic receptor expression in peritoneal macrophages was evaluated by flow cytometry.

Results: The program of habitual exercise increased the activity of peritoneal macrophages in both obese and lean animals. Terbutaline decreased phagocytic and microbicide activity, as well as the percentage of phagocytic macrophages, in both obese and lean mice. However, the exercise abolished the inhibitory effect of terbutaline, without impairing these capacities in both experimental groups. Macrophages from obese animals expressed higher levels of β 2

adrenergic receptors than those from lean animals, but regular exercise decreased the expression of this receptor in both experimental groups.

Conclusions: Regular exercise decreases the expression of β_2 adrenergic receptors in peritoneal macrophages, thus preventing, in exercised animals, the β_2 agonist-induced decrease in phagocytic and microbicide capacity of peritoneal macrophages. This is especially relevant in exercise-induced anti-inflammatory effects mediated by noradrenaline, in order to avoid a decline in the innate immune response against pathogen challenge; particularly in obese subjects who present an immunocompromised status.

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Obesity, exercise, macrophages, β_2 -adrenergic receptor, inflammation

P1-27

INFLUENCE OF OBESITY AND HABITUAL EXERCISE ON THE β_2 ADRENERGIC REGULATION OF PHAGOCYtic AND MICROBICIDE CAPACITY OF CIRCULATING MONOCYTES FROM C57BL/6J MICE

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Introduction: Obesity is a worldwide epidemic associated with comorbid conditions that involve alterations of the innate immune response and changes in the function of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. Catecholamines secreted by the sympathetic nervous system and the adrenal glands are important immunoregulatory molecules, and adrenergic agonists interfere with the inflammatory response. However, the influence of obesity on the adrenergic regulation of the innate/inflammatory response (particularly in monocytes), and the influence of regular exercise on the mechanisms underlying this regulation in obesity, has been not studied yet. **Objective:** The aim was to examine the influence of obesity, and a program of habitual exercise in this condition, on the β_2 -adrenergic regulation of the phagocytic and microbicide capacity of circulating monocytes from C57BL/6J mice.

Methods: A group of 24 eight-week old C57BL/6J mice were fed a diet containing either 5% (lean group, n=12) or 40% of fat (obese group, n=12) for 18 weeks. After 10 weeks, 6 animals from each group were subjected to a habitual exercise program (running, 3 days/week for 45 min at 18 m/min for 8 weeks). The phagocytic and microbicidal capacity of circulating monocytes against opsonized bacteria was evaluated by flow cytometry in whole blood, in the presence or absence of the β_2 adrenergic agonist terbutaline.

Results: Terbutaline did not affect phagocytic or microbicidal capacity in lean mice, whereas it decreased these capacities in obese mice. The program of habitual exercise abolished the inhibitory effects of terbutaline on the phagocytic and microbicidal capacities in obese mice, even significantly increasing microbicidal activity as measured by the oxidative burst.

Conclusions: In obese animals, regular exercise prevents the β_2 adrenergic-induced decrease in the phagocytic and microbicidal capacities of monocytes. This is especially relevant in exercise-induced anti-inflammatory effects mediated by noradrenaline, in order to avoid a decline in the innate immune response against

pathogens; particularly in obese subjects who often present an immunocompromised status.

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Obesity, exercise, monocytes, β_2 -adrenergic regulation, inflammation

P1-28

REDUCTION IN MACROPHAGES FORMING CROWN-LIKE STRUCTURES IN THE WHITE ADIPOSE TISSUE OF C57BL/6J OBESE MICE AFTER REGULAR EXERCISE

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Introduction: The combination of a sedentary lifestyle and poor eating habits has led to a dramatic rise in obesity rates around the world. Visceral fat accumulation and macrophage infiltration of white adipose tissue (WAT) are associated with low-grade chronic inflammation and obesity-related metabolic complications such as insulin resistance. The majority of macrophages in WAT can be found aggregated, forming “crown-like structures” (CLS) surrounding dead (necrotic-like death) adipocytes, a pathologic hallmark of obesity. Regular exercise is a widely used strategy in the prevention and treatment of obesity, and several immune/inflammatory mechanisms are implicated in the effects of this strategy. However, the influence of regular exercise on the presence of CLS is yet to be clearly elucidated.

Objective: The aim of this investigation was to determine the influence of obesity, and a program of regular exercise in this condition, on the prevalence of macrophages forming crown-like structures in the white adipose tissue of C57BL/6J mice.

Methods: A group of 16 eight-week old C57BL/6J mice were fed a diet containing either 5% (lean group, n=8) or 40% of fat (obese group, n=8) for 18 weeks. After 10 weeks, 4 animals from each group were subjected to a habitual exercise program (running, 3 days/week for 45 min at 18 m/min for 8 weeks). Presence of CLS in the WAT of the animals was evaluated by immunohistochemistry, using fluorescent staining with DAPI and specific antibodies for macrophages. CLS were quantified under fluorescence microscope.

Results: Obese mice presented a significantly higher number of CLS than lean mice, in which these structures were hardly present. Obese mice subjected to the program of regular exercise showed a lower number of CLS, whereas, paradoxically, lean mice subjected to the exercise presented a greater number of CLS.

Conclusions: Obesity induces a pro-inflammatory status in WAT reflected by a higher presence of CLS which can be reversed by regular exercise without diet, at least partially. This anti-inflammatory effect is opposite to that found in the control lean animals, in which exercise stimulates the inflammatory response, also reflected in the present study by an increased presence of CLS in WAT.

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Obesity, exercise, macrophages, adipose tissue, inflammation

P1-29

BENEFICIAL EFFECTS OF AEROBIC AND RESISTANCE COMBINED TRAINING ON INFLAMMATORY SIGNALING PATHWAYS AND GUT MICROBIOTA IN OBESE PEDIATRIC PATIENTS

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The severity and frequency of childhood obesity has increased significantly over the last decades, becoming in a serious and urgent public health problem. This condition is due to several factors as high-fat diet and physical inactivity, but it is also related to an increase on proinflammatory biomarkers and microbiota alterations. Exercise is an effective means for prevention and treatment of childhood obesity. We aim to assess the effect of a 12-week combined training on the inflammatory signaling pathways, gut microbiota and associated metabolic outcomes (body composition, serum inflammatory markers, lipid profile, and fasting glucose and insulin concentrations) in otherwise healthy children with obesity.

Method: Twenty-seven obese pediatric patients were randomized to a training (TG) or a control (CG) group. TG performed a 12-week combined aerobic and resistance training program, while CG followed their daily routines. Peripheral blood mononuclear cells were isolated from blood samples obtained before and after the intervention, and gene expression of proteins involved in inflammatory status and inflammation-related pathways were analyzed. The composition of gut bacterial communities was determined by 16S ribosomal RNA metagenomic sequencing.

Results: At baseline and after the intervention, there were no significant differences between TG and CG in anthropometric and biochemical parameters. Respect to the inflammatory signaling pathways, a 12-week combined training program decreased the protein levels of TLR4 (-12% vs baseline), NLRP3 inflammasome (-24% vs baseline) and protein expression of caspase-1 (-23% vs baseline) and procaspase-1 (-21% vs baseline). Osteopontin, a proinflammatory cytokine with a key role in the development of adipose tissue inflammation and insulin resistance, also decreased (-20% vs baseline) in response to exercise. On the other hand, the microbial composition at phylum level in obese patients showed an increase in the relative percentage of Bacteroidetes and Proteobacteria phyla, while Firmicutes and Actinobacteria phyla exhibited an opposite pattern in comparison to a group of healthy non-obese controls. Exercise reverted partially these changes showing a significant rise in Proteobacteria detection.

Conclusion: Data suggest that exercise decreases the main inflammatory signaling pathways induced by obesity in children and is capable to modulate infant gut microbiota. However, more evidence is needed to understand the molecular mechanisms involved in the protective effect of the exercise and the crosstalk between inflammation and gut microbiota.

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Childhood obesity, gut microbiota, inflammation, exercise

P1-30

THE USE OF SOCRATIVE AS AN EVALUATION TOOL FOR LABORATORY LESSONS IN PHYSIOLOGY: STUDENTS OPINION AND ACADEMIC PERFORMANCE OBTAINED

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Introduction: The emergence of new informative and communication technologies and the increasing interest among young people for these technologies made us to consider that the use of a mobile application could be useful to evaluate the competences acquired by the students. We developed a training innovation project in which a smartphone application (www.socrative.com) was used to evaluate the skills and competences acquired by the students at the Physiology, Physiopathology and Cell Biology laboratory lessons. This tool has certain advantages compared to others such as is free, easy to use for both teacher and students and, as every student has a screen, it allows the incorporation of high-quality images to the test.

Methodology: On completion of the project, the student's opinion regarding this new evaluation system was analyzed and the academic performance improvement was evaluated by comparing the result with previous course.

Results: 70% of the students were satisfied with the new evaluation system and found it a straightforward and user-friendly set. However, almost half of the students did not consider that the tool improves the evaluation system, and others did not rely on the application and felt more confident with the traditional one. Interestingly, 70% of the students think that it may be useful during theoretical lessons. Globally, no major differences were observed in the calculations between the students that have employed the new evaluation system and those using the previous. However, when the data were analyzed in terms of subject it was found that in Cell Biology (belonging to the 1st course; without any prior knowledge of the tool) the failure rate increased significantly, while in the subject Physiopathology (belonging to the 3rd course; with some prior knowledge) it decreased.

Conclusion: According to the student's opinion, although this evaluation system does not have great improvements when compared with the traditional one, it could be a strong tool so as to consolidate the acquired skills and/or to close remaining gaps during the theoretical lessons. The use of this smartphone application for the evaluation only improves the academic performance in those students with some prior knowledge of the tool.

Socrative, physiology innovation project, smartphone application

P1-31

LOWER GASTROINTESTINAL BLEEDING: THE SILENT HEMODYNAMIC COMMITMENT. PHYSIOPATHOLOGICAL STUDY OF THE DISEASE AND PRACTICES FOR STUDENTS ON DECISION MAKING AND PROBLEM SOLVING

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Introduction: Digestive hemorrhages are a frequent cause of medical emergency. Upper gastrointestinal bleeding is very notorious, serious and life threatening, making patients to consult immediately the doctor. Lower Gastrointestinal Bleeding (LGIB), manifests as hidden blood in stool, leading many times to a medical consultation for asthenia and fatigue, delaying a potential diagnosis. **Objective:** We aim to develop simulated scenarios of LGIB to many different degrees, from insidious blood loss to large hemorrhages that compromises normal hemodynamic equilibrium. During scenarios, students have to perform decisions and develop skills for treatment. 90% of the cases are mild, 10% lead to hypovolemic shock.

Methods: During the course of LGIB there might be important hemodynamic compromises. In order to reproduce this pathology, we employed the Human program, where we set blood loss of 1500 ml for 8 hours to days, showing different levels of increased cardiac frequency, reduction of the blood pressure, while blood volume and central venous pressure (CVP) are reduced. We kept track of many compensatory mechanisms including aldosterone, erythropoiesis, etc. For treatment we simulate blood transfusion.

Results: The time course of LGIB is shown as vital signs and physiological variables. Difficulties arises when students face the severity and consequently have to decide action. Trying to solve that problem is possible to use analytics and advanced imaging tests, such as CAT or delayed colonoscopy, so as to identify the bleeding point and decide the need for urgent endoscopic cauterization or assume an expectant attitude if admission in hospital is required.

Conclusions: Not all pathologies are easily detected, such in this LGIB condition, this is why students need to understand the value of indirect signs to guide their clinical history. Precise guidance and signs of evolution are critical to understand this pathology.

Lower-Gastrointestinal-Bleeding; Medical-Simulation; Hemodynamic-Compromise; Advanced-Teaching

P1-32

LEARNING PHYSIOLOGY WITH COMPUTATION MODELS: CARDIO-RESPIRATORY ADJUSTMENTS DURING HEMORRHAGE

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Practical case simulation in Medical Degree is widely used as a complement to master class teaching in the classroom. This method provides the opportunity to learn Cardiocirculatory and Respiratory Physiology by simulation experiences in Human Physiology. We use a Simulation Laboratory located at the own Valladolid University medical school equipped with a simulator manikin (iSTAN, Medical Simulator, Mianz, Germany), and the associated software (MUSE) intended to improve medical student learning. The manikin mimics breath sounds, heart tones and palpable pulses providing real-time information to students. Using computation models, we are able to recreate real situations that cannot be executed any other way. The manikin consists of a monitor displaying EKG, pulse oximeter, blood pressure, arterial and pulmonary wave forms, etc. The MUSE software is versatile and very integrative. It requires tuning simulated practical examples to acquire competences and skills in Physiology and to promote student's reflective thinking. Our aim has been to implement practical cases in MUSE, using as a reference the physiological variables obtained in the web HUMAN, a MS-DOS human

physiology model. Throughout several courses, we have elaborated several practical examples that include acute myocardial infarct, rest in moderate and intense hypoxia, moderate and intense exercise in normal atmosphere and high altitude. Here, we present the simulation of a hemorrhage, the parameters progress over time and their physiological adaptations. We focus on cardiovascular and respiratory parameters: evolution of the mean arterial blood pressure, systolic, diastolic and pulse pressure, heart rate, cardiac output, temperature, oxygen consumption, SatO2 and hematocrit. We have obtained the relationships between heart rate, blood pressures and respiratory rate. Thus, we can easily observe how the circulatory and respiratory variables are modified. From the analysis of these variables, students can generate an overall picture of how the organism responds to moderate and/or intense hemorrhage. At the end of the simulation, the students are invited to think over on causes and mechanisms for the observed cardio-respiratory adjustments, the implications of baroreflex and chemoreflex and, finally, the effect of a blood infusion. We conclude that the use of manikin simulation and MUSE software is potentially useful for linking the theoretical fundamentals and the practical teaching of physiology. It reinforces self-learning. It also serves an auto-evaluation function, as it includes multiple-choice questions. Finally, it is safe for the patients as it does only involve virtual ones.

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Physiology, Hemorrhage, Medical Simulator, MUSE

P1-33

EDUCATION IN RESEARCH ABILITIES IN PHYSIOLOGY AND MEDICINE: LONGITUDINAL ANALYSIS OF THE IMPACT OF A CORE COURSE IN “BIOMEDICAL RESEARCH AND NEW TECHNOLOGIES”

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That Medicine is sustained on research-generated knowledge seems an obvious statement, and teachers of Physiology courses often take the responsibility of transmitting research abilities to future physicians in a transversal way. In our university, the course “Biomedical Research and New Technologies” implanted after switching to EHEA (“EEES”) plan, has naturally convened Physiology teachers as drivers of this new enterprise. The global aim of this course is to provide Medical Graduates with a solid education in biomedical research, focusing on practical research abilities to be applied, first during their undergraduate years (either as Research Interns, many times associated to Physiology Departments, or in their “End-of-Degree Project”), and later during their ongoing education as Medical Interns. The course covers general concepts in research, its methods, and evidence-based medicine. It also covers practical abilities in data mining, critical analysis and communication of research results, critical appraisal, reference managing, image analysis, and quantitative data analysis. Our Teaching Team is carrying out an “Innovation in Teaching Research Project” to assess the long-term impact of this course in Medical graduates. We designed a series of web-based screening tools to evaluate (i) the appreciation of research by students, (ii) their use of research-related tools, and (iii) their competence in research-related tasks. These three domains are explored before they take the course, during their 6th year of Medical School, and during the 1st year of Medical Internship. We have collected a longitudinal series (2015–2018), covering graduates

from the previous academic program, not including formal education on research, graduates from the new system, and medical interns from the two Valladolid University Hospitals. We have also started a comparative analysis with other Universities (UAM, Madrid) to evaluate the outcome of diverse academic programs. Our results depict a very interesting scenario. Students entering their second year of Medicine show quite a high interest in research, while those graduating from the previous academic program reveal important deficiencies, revealing needs that were not covered in their education. Data from Medical Graduates that have experienced the program show clear improvements, both conceptual and ability-related, making the effort of introducing courses of this type, where formal education on research is undertaken, worthwhile. However, they also indicate that more effort is needed if we want to promote to the generation of Medical Doctors with a solid research education, with the foreseen beneficial consequences for the quality and excellence of our Healthcare System.

Biomedical research, education outcome, research training

P1-34

DEVELOPMENT OF RESEARCH SKILLS IN PHYSIOLOGY AND MEDICINE: COMPARATIVE ANALYSIS BETWEEN PUBLIC UNIVERSITIES

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Physiology educators in Biomedical Degrees consider research an essential part of the curriculum. However, not many Spanish universities include specific courses to develop research abilities. The Schools of Medicine of “Universidad Autónoma de Madrid” (UAM) and “Universidad de Valladolid” (UVa) have incorporated the courses “Initiation to Biomedical Research” and “Biomedical Research and New Technologies”, respectively in their curricula. Their aim is to provide Medical Graduates with a solid education in basic biomedical research, focusing on practical research abilities, including scientific literature search, data management, analysis, interpretation, and critical appraisal of scientific data. Basic science departments, including Physiology department, are highly committed with these courses, tutoring students and ensuring that they acquire research-related competences. Physiology lecturers of UAM and UVa have joined efforts to evaluate the knowledge of the students about Science and their biomedical research competences before and after participating in these courses. In the present work we aim to assess, at the beginning of the degree, the following aspects: (i) the appreciation of research by students, (ii) their use of research-related tools, and (iii) their competence in research-related tasks. We also aimed to compare the results in these three domains between UAM and UVa. We have designed a specific questionnaire based on Likert and binary scales, combined with exercises such as graph interpretation. A total of 225 students from UAM and 229 from UVa participated in the study. Our analysis of the first domain reveal that students have a positive perception of scientific knowledge and recognize that Science is important for the progress of knowledge and it is useful for their future profession. However, knowledge of research-related tools and scientific skills are scarce at this stage of their education; this deficit is related to their difficulties in the interpretation of graphically presented data. We also evidence marked similarities between both universities in the student profile regarding the three assessed domains. In conclusion, we have detected important education needs, both conceptual and ability-

related, in the research education of our future physicians in both universities. Our future goal is to assess if the Biomedical Research courses have a positive impact, covering those education needs.

Biomedical research teaching

P1-35

PRELIMINARY EVALUATION OF THE IMPLEMENTATION OF AN APP FOR VIRTUALIZED LEARNING OF SCIENTIFIC AND MEDICAL TERMINOLOGY

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Increasing progressive deterioration of the comprehension and use of the medical and scientific terminology by the students have been observed. University education nowadays generally neglects the transmission of these skills. The direct consequences of this situation is a poorly effective and precise communications when the student becomes a professional, which, in turn, has a negative impact on their employability and professional performance. The new pedagogical trends reinforce the use of virtualization and digitalization as a way to achieve maximum profitability of the formative process. The “gamification” is an innovative tool to encourage student self-learning in an attractive and dynamic manner. A virtual tool consisting in a mobile app, “Exprésate con Ciencia”, was developed in order to promote autonomous learning of students. Objective The objective of this study was to evaluate in a preliminary way which have been the main channels of diffusion of the tool, the diffusion magnitude of the app, as well as the evaluation of the users of the university environment

Methodology: Through a form available from google docs, 76 students were surveyed. The questions made reference to the utility of the tool versus other classic formats, the simplicity of use and the attractive and innovative character. On the other hand, proposals were requested in order to improve the tool in the future. Finally, questions about the way in which the user got to know the app were included.

Results: This app published in February 2018 for Android and iOS platforms has been downloaded a total of 467 times (342 from Android and 125 from iOS systems). Regarding the utility of the app by the users, more than 80% of the users considered this app as good or very good. Furthermore, the same percentage of users pointed that they prefer using the app instead educational material on paper and considered easy to use. In order to improve the app, the user proposed to divide the contents by area of knowledge. Also, the app has been downloaded by a wide range of

the population. Undergraduate students, PhD and professors are users of this app, being the majority of the users graduate students of Pharmacy, Nutrition and Biotechnology degrees. Finally, the main channel of diffusion of the tool was through peers.

Conclusion: In this preliminary evaluation, the results demonstrate the usefulness, accessibility and attractiveness of the virtual tool developed for the learning of scientific and clinical terminology.

App, learning, scientific, terminology, gamification

P1-36

BEHAVIORAL PROTOCOL FOR THE STUDY OF COOPERATION IN MICE

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Animals can learn and accomplish different tasks thanks to their cognitive capabilities. In particular, instrumental learning has been used in many studies involving different animal models. In the same way, cooperative behaviors can be observed in many species while getting natural resources, mostly in cases when they will not be able to obtain them individually. The main aim of our research was the acquisition of an operant conditioning task by cooperating laboratory mice (*Mus musculus*, strain C57Bl/6). Relationship between animal models and environmental factors related to animal housing conditions were analyzed. The influence of modifying dopamine receptors with reuptake inhibitor and antagonistic substances like methylphenidate and haloperidol, respectively, was also analyzed to know their effects on the ability to perform the designed tasks. We developed a protocol as well as an experimental box for studying the coordinated ability, i.e., cooperative behaviors to organize temporo-spatial actions to achieve a common goal. Experiments were divided in four phases: individual learning was analyzed in the first and second phases and learning in pairs in the third and fourth ones. Experimental boxes ($n = 4$) had an igloo and auto-food dispenser which was opened when an animal (or a couple of them) went into the igloo, depending on the phase. Our results show the validity of the experimental protocol for studying instrumental learning, and the adaptation of the animal model used for the task, individually and in pairs. With regard to environmental factors, a positive effect was observed when mice lived in the animal house in pairs before the experiment, according to its weight and activity levels. As a result, frequency and severity of fights were reduced during pairing phases. Finally, our results show an influence, positive or negative depending on the substance used, on the learning efficacy when dopamine levels are modified, with short-term and reversible effects after stopping administration. Currently, we are studying the importance of vicary learning in cooperative behavior in mice.

Behavior, cooperation, mice, methylphenidate, haloperidol

P1-37

DECISION-MAKING IN A GO/NO-GO TASK BY VISUAL-STIMULI SHOWN IN IPAD IN RATS

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Go/No-Go tasks allow to assess decision making processes and, in addition, the ability to suppress a specific action according to the context, or

the ability to cancel an action in progress when an unpredictable cue indicates that it should be avoided. In many of these experimental procedures, food is used as a positive reinforcement to study learning processes or decision-making abilities. In the present experiments, rats had to discriminate between two visual stimuli shown in an iPad (Go or No-Go) disposed in horizontal or vertical way and in white or green color. In this situation, the execution (Go) or non-execution (No-Go) of the selected action (to touch or not the visual display) would be reinforced. Rats were trained in a modified Skinner box equipped with an iPad, where stimuli appeared. The main goal was to record and analyze the local field potentials (LFPs) collected from cortical and subcortical brain structures, when the visual stimuli were shown in the iPad screen and during the subsequent activities. Animal behavior was videotaped and quantified. When a tone sounded, the rat had to press a white horizontal rectangle (Go) and to avoid touching the green vertical one (No-Go), getting in this way a pellet as reward. Previously, rats had gone through caloric restriction and maintained to 85–90% of their initial weight. The experiment consisted of five phases. Firstly, rats learned to approach the iPad and touch the stimulus in the case of Go trials as well as not to approach it in No-go trials. In the next phases, stimuli were mixed in an increasing way (Go, No-Go, 50%, 25%, and intermingled). Rats were implanted with recording electrodes in motor cortex, prelimbic cortex, accumbens nucleus core, hippocampus, mediodorsal thalamic nucleus, basolateral amygdala, dorsolateral and dorsomedial striatum. Each animal was implanted with recording electrodes in five of the indicated sites. These brain areas are involved in cognitive and motivational processing, execution of motor responses, and contribute to reward-directed behaviour. Preliminary results indicate that rats are able of acquiring this rather complicate task with a significant performance even when stimuli were mixed at a 25%. Spectral analyses of collected LFPs is being carried out.

Go/ no Go, iPad, reward, LFP

P1-38

ROLE OF EXPERIMENTALLY INDUCED TRANS-SYNAPTIC LONG-TERM POTENTIATION

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Long-term potentiation (LTP) evoked by high frequency stimulation (HFS) is a very well-known experimental procedure that shares certain mechanisms with learning and memory processes. LTP is a typical example of synaptic plasticity, which appears after applying an HFS train to the afferent pathway of a CNS synapse. Basically, the LTP consists in an increase of the synaptic response to a control stimulus following the presentation of the HFS train. This technique was described for the first time in the hippocampus and it is still studied mostly there, since those synaptic connections are very susceptible to evoke LTP after HFS presentations. Although most of these studies have been performed in vitro, we have developed a new experimental approach to carry out those experiments in behaving animals. In the present study, field excitatory post-synaptic potentials (fEPSP) evoked at five different synapses of the hippocampal circuit were selected. Experiments were carried in alert behaving mice. The main goal in this work was to ensure that there are synaptic changes in strength not only in the synapse where LTP was induced, but also in those synapses which are contiguous to it. To that end, fEPSPs evoked in five hippocampal synapses were studied in mice, in both ipsi- and contralateral hemispheres. HFS were presented to the perforant pathway (PP) and recordings were carried out in the CA1 and CA3 areas of the ipsilateral hippocampus and in the CA1 area of the contralateral side. The five studied synapses were: PP-CA1i, PP-CA3i, PP-CA1c, CA3-CA1i, and CA3-CA1c. Animals were prepared for chronic recordings

following procedures described elsewhere (Gruart et al., J. Neurosci., 2006). We have characterized input/output curves, pair pulse facilitation (PPF) and LTP of these five synapses. Data from input/output curves and paired-pulse facilitation proved that the five studied synapses have similar basic properties, which makes their later comparison easier for subsequent histological, pharmacological, and genetic manipulations. Importantly, following HFS of the PP, we observed the presence of significant LTP both at the CA3-CA1c and PP-CA1c synapses. In conclusion, these preliminary results clearly indicated that LTP can be evoked at synapses located far away from the stimulated afferent pathways.

Trans-synaptic LTP, synapse, HFS, hippocampus

P1-39

NITRIC OXIDE AS A FEASIBLE MEDIATOR IN POSTNATAL SYNAPTIC REMODELING IN MOTONEURONS

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Maturation of the central nervous system involves synaptic rearrangement during postnatal development. However, knowledge of molecular mechanisms underlying such a process is still incipient. Nitric oxide (NO), a highly reactive gas synthesized by NO synthase (NOS), has been recognized as a mediator in synaptic remodeling during embryonic development. In particular, a transient expression of NOS occurs in motoneurons of neonatal rats that disappears three weeks after birth (P21). Interestingly, postnatal maturation of hypoglossal motoneurons (HMNs) involves a loss of GABAergic synaptic inputs, while the number of glutamatergic ones remains unaltered. In this way, our group recently demonstrated that NO induces the withdrawal of synaptic buttons by phosphorylation of myosin light chain (p-MLC), which is known to trigger actomyosin contraction and neurite retraction. Thus, the aim of this work was to study the involvement of NO on synaptic remodeling on HMNs during postnatal development. For that, osmotic micropumps, filled with the NOS inhibitor N^w-nitro-L-arginine methyl ester (L-NAME) or its inactive stereoisomer D-NAME, were subcutaneously implanted in neonatal rats at P7. Drug concentration was adjusted to assure a chronic delivery of 180 mg/kg/day for 14 days. Coronal sections (30 μm-thickness) of brainstem, obtained from animals perfused at P21, were processed by immunohistochemistry against VGLUT2, to identify glutamatergic excitatory inputs; VGAT, to detect GABAergic inhibitory inputs, and SMI32 as a marker of motoneurons. In L-NAME-treated pups, analysis by means of confocal microscopy reported a reduction ($-13.5 \pm 1.1\%$; $n = 193$ HMNs) in the excitatory and an increase ($+28.7 \pm 3.1\%$; $n = 31$ HMNs) in the inhibitory synaptic coverage of HMNs relative to D-NAME-treated animals. Furthermore, western blotting analysis of microdissected hypoglossal nuclei, extracted from P7, P10, P18 and P21 rats, revealed a progressive increase in levels of p-MLC from P18 ($+34.3 \pm 20.8\%$; $p < 0.05$, Mann-Whitney U test) that was greatly accentuated at P21 ($+172.7 \pm 28.1\%$; $p < 0.01$, Mann-Whitney U test). Interestingly, L-NAME reduced drastically p-MLC levels ($-49.5 \pm 9.7\%$; $p < 0.01$, Mann-Whitney U test) in the hypoglossal nucleus at P18 relative to the D-NAME-treated group. Altogether, these results suggest that physiological NO synthesis during early postnatal development acts as a synaptotrophic agent for glutamatergic inputs and a synaptotoxic factor for the inhibitory ones. The mechanism by which NO acts seems to involve phosphorylation of MLC, which, in turn, could modulate actomyosin contraction and, subsequently, mediate synaptic rearrangement.

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Synaptic remodeling, NO, VGLUT2, VGAT, MLC

P1-40

RHO-KINASE ALPHA CONTROLS INSPIRATORY-RELATED ACTIVITY OF HYPOGLOSSAL MOTONEURONS IN THE ADULT RAT

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Rho-associated kinase (ROCK) regulates neural cell migration, proliferation and survival, dendritic spine morphology, axon guidance and regeneration, and synaptic function. However, whether this kinase modulates neuronal discharge activity has been poorly investigated so far. Several findings support this hypothesis: i) ROCK regulates several ionic channels, among them, the leak potassium channel TASK1, highly expressed in motoneurons; ii) both ROCK alpha and ROCK beta isoforms are expressed in the hypoglossal motor nucleus (HN) of neonatal rats; iii) ROCK alpha, in particular, is highly expressed by hypoglossal motoneurons (HMNs); and iv) ROCK alpha regulates intrinsic membrane excitability of motoneurons in vitro. In this line, here we aimed to unveil ROCK as a regulator of the inspiratory-related discharge pattern of HMNs in the adult rat. In the decerebrated, vagotomized and paralyzed adult Wistar rat, at baseline (end-tidal CO₂: 4.8–5.2%; O₂: 15–16%), unitary extracellular recording of antidromically-identified HMNs displayed bursts of action potentials (spikes, sp) synchronized with the inspiratory phase of breathing. Then, we analyzed mean firing rate (mFR), peak FR (pFR), duration (DB), and number of spikes (SB) per burst of HMNs in untreated animals, and 48–72 h after an unilateral injection (1 microliter) in the HN of a small interfering RNA directed against ROCKalpha (siRNAalpha) or ROCKbeta (siRNAbeta), or a non-targeting siRNA (cRNA) as the control condition. Strikingly, siRNAalpha, but not siRNAbeta, induced a significant ($p < 0.05$, student t-test) reduction in mFR (30.7 ± 2.7 sp/s), pFR (92.3 ± 12.8 sp/s) and SB (15.8 ± 1.7 sp) per burst as compared with the cRNA-treated (mFR: 59.5 ± 5.8 sp/s; pFR: 192.6 ± 13.4 sp/s; SB: 25.1 ± 2.4 sp) or untreated (mFR: 56.4 ± 3.1 sp/s; pFR: 190.5 ± 9.5 sp/s; SB: 28.9 ± 3.1 sp) conditions. However, no differences were detected between groups for DB, and, interestingly, for burst rate, indicative of the integrity of premotor structures. Altogether, these outcomes indicate that ROCKalpha is necessary for the normal performance of inspiratory-related activity of HMNs.

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ROCK, motoneuron, electrophysiology, breathing

P1-41

DORSOLATERAL PERIAQUEDUCTAL GREY MATTER AND PONTINE A5 REGION CONNECTIVITY: A NEUROPHARMACOLOGIC AND ELECTROPHYSIOLOGICAL STUDY

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In this study, carried out in spontaneously breathing anaesthetised rats, we have analysed the relevance of the interactions between the dorso-lateral periaqueductal grey matter (dlPAG) and the A5 region, and how this sympathetic pontine region participates in modulating the cardiorespiratory response evoked from the dlPAG. Electrical stimulation of the dlPAG (1 ms pulses, 20–30 μ A given at 100 Hz for 5s) was elicited, and the evoked cardiorespiratory changes were analysed before and after ipsilateral microinjections of muscimol (50 nl, 0.25 nmol, 5s) within the A5 region. dlPAG stimulation evoked the classical “defence response” characterized by tachypnoea, hypertension and tachycardia. Tachypnoea consisted of an inspiratory facilitatory response [increase in respiratory rate ($p < 0.001$) due to a decrease in expiratory time ($p < 0.01$)] and was accompanied by a pressor ($p < 0.001$) and tachycardic ($p < 0.001$) response. Microinjection of Muscimol within the A5 region reduced all, pressor ($p < 0.05$), heart rate ($p < 0.001$) and respiratory ($p < 0.001$) responses evoked by electrical stimulation of dlPAG. Finally, extracellular recordings of putative A5 neurones were obtained during dlPAG electrical stimulation in order to assess functional interactions between A5 and dlPAG. Forty A5 cells were recorded, 16 of which were affected by dlPAG (40%). With these results, we can conclude that neurones of the A5 region possibly modulate the cardiorespiratory response evoked from the dlPAG.

A5 Region, dlPAG, cardiorespiratory control, rat

P1-42

NMDA RECEPTORS CONTAINING GLUN2B/2C/2D SUBUNITS MEDIATED AN INCREASE IN GLUTAMATE RELEASE AT HIPPOCAMPAL CA3-CA1 SYNAPSES

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NMDA receptors (NMDARs) are involved in synaptic transmission and synaptic plasticity in different brain regions, and they modulate glutamate release at different presynaptic sites. Here we studied whether non-postsynaptic NMDARs, putatively presynaptic (preNMDARs), are tonically active at hippocampal CA3-CA1 synapses, and if they modulate glutamate release. We found that when postsynaptic NMDARs are blocked by MK801, D-AP5 depresses evoked and spontaneous excitatory synaptic transmission (evoked: $67 \pm 7\%$, $n=7$ and spontaneous: baseline 0.33 ± 0.01 Hz, $n=7$ vs DAP-5 0.22 ± 0.01 Hz, $n=7$), indicating that preNMDARs are tonically active at CA3-CA1 synapses, facilitating glutamate release. The subunit composition of these NMDARs was determined by studying evoked and spontaneous excitatory synaptic transmission in the presence of Zn^{2+} , Ro 25-6981 and PPDA, antagonists of NMDARs containing GluN2A, GluN2B and GluN2C/D, respectively. We found that evoked and spontaneous release decreased when the activity of NMDARs containing GluN2B (evoked Ro 25-6981 $69.8 \pm 8\%$, $n=6$; spontaneous: baseline 0.39 ± 0.02 , $n=6$ vs Ro 25-6981 0.24 ± 0.01 Hz, $n=6$) and GluN2C/D (evoked PPDA $63 \pm 6\%$, $n=6$; spontaneous: baseline 0.41 ± 0.02 Hz, $n=6$ vs PPDA 0.26 ± 0.02 Hz, $n=6$) subunits but not GluN2A (evoked: $104 \pm 3\%$, $n=6$; spontaneous: baseline 0.40 ± 0.05 ,

$n=7$ Hz vs Zn^{2+} 0.41 ± 0.07 Hz, $n=7$) was impeded. In addition, we found that the increase in glutamate release mediated by these NMDARs requires protein kinase A (PKA) activation (evoked treated with H-89 and subsequent application of DAP-5: $102 \pm 7\%$, $n=7$ vs non-treated slices $67 \pm 6\%$, $n=6$; spontaneous: baseline 0.38 ± 0.01 Hz, $n=7$ vs DAP-5 0.41 ± 0.03 Hz, $n=7$). We conclude that preNMDARs that contain GluN2B and GluN2C/2D subunits facilitate glutamate release at hippocampal CA3-CA1 synapses through a mechanism that involves PKA.

Patch-clamp, NMDA receptor, hippocampus, pharmacology

P1-43

ADENOSINE RECEPTOR-MEDIATED DEVELOPMENTAL LOSS OF SPIKE TIMING-DEPENDENT DEPRESSION IN THE HIPPOCAMPUS

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Critical periods of synaptic plasticity facilitate the reordering and refining of neural connections during development, allowing the definitive synaptic circuits responsible for correct adult physiology to be established. Presynaptic spike timing-dependent long-term depression (t-LTD) exists in the hippocampus, which depends on the activation of NMDARs and that probably fulfills a role in synaptic refinement. This t-LTD is present until the 3rd postnatal week in mice, disappearing in the 4th week of postnatal development. We were interested in the mechanisms underlying this maturation related loss of t-LTD and we found that at CA3-CA1 synapses, presynaptic NMDA receptors (preNMDARs) are tonically active between P13 and P21, mediating an increase in glutamate release during this critical period of plasticity. Conversely, at the end of this critical period (P22–P30) and coinciding with the loss of t-LTD, these preNMDARs are no longer tonically active. Interestingly, this t-LTD can be completely recovered by antagonizing adenosine type 1 receptors (A1R), which also recovers the tonic activation of preNMDARs at P22–P30. By contrast, the induction of t-LTD was prevented at P13–P21 by an agonist of A1R, as was tonic preNMDAR activation. Furthermore, we found that the adenosine that mediated the loss of t-LTD during the fourth week of development is supplied by astrocytes. These results provide direct evidence for the mechanism that closes the window of plasticity associated with tLTD, revealing novel events probably involved in synaptic remodeling during development.

Adenosine, astrocytes, hippocampus, synapse, plasticity

P1-44

GENETIC EXPRESSION OF ARC AND C-FOS IN THE INSULAR CORTEX IS RELATED WITH TASTE FAMILIARITY

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The taste neophobic response reflects the reluctance to ingest novel tastes in order to avoid negative consequences. Several studies have

demonstrated the involvement of the insular cortex (IC) in the taste neophobic response (Lin et al., 2015; Lin and Reilly, 2012; Moraga-Amaro et al., 2014; Adaikkan and Rosenblum, 2015). Discriminating novel and familiar taste stimuli is very important for survival. Therefore, taste familiarity is memory-dependent. Consolidation of long-term memories requires de novo protein synthesis induced by the activation of immediate early genes that act as messengers. Thus, the level of gene transcription is coupled with changes in the protein synthesis and neuronal activity. The main aim of the present research is to explore both the behavioral performance and the expression profile of different immediate-early transcription factors (*arc*, *jun*, *c-fos*, *narp* and *zif-268*) in the insular cortex after exposure to a saccharin solution (0.4%) during the first (novel), the second (familiar I) and the sixth presentation (familiar II). The period of six days was chosen in order to obtain a complete attenuation of taste neophobia. We have found increased expression of *arc* and *c-fos* gene 30 min in the familiar I after drinking a saccharin solution which was becoming familiar during the second presentation in comparison with the group Novel that received the first exposure. No changes in the expression of *zif-268*, *jun* and *Narp* genes were found. The results support a role of the insular cortex in the consolidation of taste memory which is mediated by the increased expression of *Arc*, *jun* and *c-fos* during the swift from novelty to familiarity.

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Neophobia, insular, *arc*, *c-fos*, taste memory

Poster session 2: Cardiovascular & Respiratory, Chronobiology, Endocrinology, Metabolism & Nutrition, Animal Experimentation, End-of-Degree Projects

P2-01

EVALUATION OF THE INFLUENCE OF ATHEROSCLEROTIC FACTORS ON THE REGENERATIVE PROPERTIES OF ENDOTHELIAL PROGENITOR CELLS APPLIED TO A MURINE MODEL OF CRITICAL LIMB ISCHEMIA

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Critical limb ischemia (CLI) is the most aggressive form of peripheral arterial disease which, starts by severe obstruction of blood vessels and is commonly caused by atherosclerosis, it presents as rest pain, ischemic ulceration or gangrene of the foot. Patients with CLI have a high risk of limb loss and fatal or non-fatal vascular events. Unfortunately, the symptomatic treatments are ineffective while conventional surgical revascularization is not possible in more than 50% of patients due to the high comorbidity. Therefore, there is a huge need to find alternative strategies for CLI and in this regard, cell therapy mediated by endothelial progenitor cells (EPCs) is postulated as a good alternative. Previous studies have proven their angiogenic potential, but it is still necessary potentiate them.

According to some studies, factors secreted by atheroma plaques can change the expression of proteins in EPCs ex vivo, promoting an initial response to an inflammatory environment as well as the activation and mobilization of these cells. In the present work, we tested the effect produced by human EPCs pre-incubated ex vivo with atheroma plaque (AP) secretome, in a murine model of CLI, after inducing ischemia by double ligation of the femoral artery. Changes in blood flow, as well as ischemic symptoms were evaluated on CLI mice by days 1, 7, 14 and 21 after surgery. Mice were sacrificed by day 21 and the femoral adjacent tissue was extracted for further immunohistochemistry to analyse capillary formation. The results obtained in the present approach indicate that the proposed strategy improves the action associated with EPCs. CLI mice treated with EPCs incubated with the secretome of carotid arteries complicated with AP showed higher blood flow recovery and a slower progression of CLI characteristics compared with mice treated with no stimulated EPCs and also with control animals, without cell administration. In addition, CLI mice treated with EPCs stimulated with AP secretome showed a higher number of capillaries at the end of the assay, by day 21. Finally, the results were better in the mice treated with EPCs stimulated with AP secretome showing a greater recovery (in all the analyzed values) against the damage promoted by the ligation of the femoral artery. In conclusion, stimulation of EPCs with atherosclerotic factors, prior their administration to CLI mice, enhanced the therapeutic action of these cells.

Ischemia, atheroma plaque, cell therapy

P2-02

GENDER INFLUENCE ON CAROTID BODY SENSITIZATION AND PULMONARY ARTERIAL HYPERTENSION IN CHRONIC HYPOXIC RATS

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Chronic hypoxia (CH) exposure as occurs physiologically living at high altitude or pathologically in COPD or pulmonary emphysema, produces sensitization of carotid body (CB) chemoreceptors, sympathetic and respiratory chemoreflexes, and increased hypoxic ventilatory responses and sympathetic activity. CH is also involved in pulmonary pathologies caused by sustained hypoxic pulmonary vasoconstriction and endothelial dysfunction, leading to pulmonary arterial hypertension (PAH) and right ventricular hypertrophy. PAH is a young female predominant disease, suggesting gender related differences in its development. We have hypothesized a gender related hypoxia effect on ventilatory reflex and pulmonary hemodynamics brought about by an oxygen sensing dimorphism. Therefore we studied CH effects on respiratory and pulmonary parameters from adult male and female Wistar rats exposed to a normobaric hypoxic atmosphere (11%O₂; pO₂ 70mmHg) during 14 days. CB endogenous catecholamine content (measured by HPLC-ED) was higher in CH male than in CH female rats, although the rate of synthesis increased similarly in both related to their control group. Plethysmography showed increased MV on female rats breathing hypercapnia (2810±250mL/min.kg⁻¹) compared to males (1892±65 mL/min.kg⁻¹) after CH exposure, mainly due to an increase of tidal volume. No differences were found in response to hypoxia tests. Pulmonary arterial pressure (PAP), measured by pulmonary artery (PA) catheterism, was higher in CH female rats (22.9 ±1.0 in CH vs. 11.74±0.17 mmHg in control) than in male counterparts

(17.7±0.7 in CH vs. 11.34±0.8 mmHg in control). The CH female's PAP increase correlated with an augmented Fulton Index (0.35±0.01 in CH vs. 0.29±0.01 in control) and a diminished heart rate (259±40 in CH vs. 414±11 in control). However, vascular endothelial injury, measured as relaxation to carbachol by miography, was only evident in PA from CH male. Interestingly, 17- β -estradiol promoted a greater relaxation in PA from CH male (65±4.6%) than CH female (35.6±2.7%). We conclude that 14 days of CH exposure can be a good model to study the gender influence on PAH developed by rats, maybe mediated by estrogens.

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Hypoxia, carotid body, pulmonary hypertension, gender

P2-03

AMP-DEPENDENT PROTEIN KINASE (AMPK) RELAXES INTRARENAL ARTERIES BY CALCIUM DEPENDENT AND INDEPENDENT MECHANISMS

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The adenosine monophosphate-activated protein kinase (AMPK) is a key enzyme that acts as a cellular energy sensor and during energy stress, AMPK stimulates energy production to restore homeostasis. Recently, AMPK has been identified as a potent vasodilator in resistance arteries (Schneider et al., Hypertension 2015; 66:108). AMPK is highly expressed in the kidney and is an important regulator of its energy metabolism but little is known about AMPK on renal vascular regulation. In the present study we sought to assess the mechanisms by which AMPK causes vascular smooth muscle (VSM) relaxation in renal resistance arteries. Renal interlobar arteries isolated from the kidney of male Wistar rats were mounted in a microvascular myograph placed on an inverted microscope equipped for dual excitation wavelength microfluorimetry. Arteries were incubated in the dark in physiological saline solution (PSS) with 8 μ M Fura 2-acetoxymethyl ester for 2 hours at 37°C and after loading, they were illuminated with alternating 340nm and 380nm light using a monochromator-based system. The Ratio (R) F340/F380 was taken as a measure of $[Ca^{2+}]_i$. Simultaneous measurements of $[Ca^{2+}]_i$ and tension were performed by Fura2-AM fluorescence in arteries with intact endothelium and endothelium-denuded. Then, time-response curves to 10 μ M AMPK selective activator (A769662) in the absence and presence of inhibitors of intermediate-conductance Kca channels (IKca), TRAM-34 and inhibitors of high-conductance Kca channels (BKca), Iberitoxin were evaluated in renal arteries pre-contracted with phenylephrine (Phe) or with 30mM K^+ . The AMPK selective activator A769662 produced relaxations of endothelium-intact and denuded phenylephrine pre-contracted arteries, that were accompanied by simultaneous decreases in VSM $[Ca^{2+}]_i$. However, relaxations were larger than the corresponding decreases in VSM $[Ca^{2+}]_i$ suggesting the involvement of Ca^{2+} desensitization mechanisms. AMPK activation-induced relaxations were abolished by raising extracellular K^+ which suggests that K^+ efflux and VSM hyperpolarization mechanisms are in part responsible for the AMPK relaxant effects in renal arteries. AMPK-induced relaxations were sensitive TRAM-34 in endothelium intact arteries, but these relaxations were not sensitive to Iberitoxin in endothelium-denuded microvessels thus indicating that AMPK activation might have a major effect on Kca channels expressed in endothelial cells. These results suggest that AMPK activates IKCa in renal endothelial cells which might initiate an endothelium-derived hyperpolarization (EDH) vasodilator response in renal arteries, via a mechanism that is different from its action in

VSM. Furthermore, besides VSM hyperpolarizing mechanisms, Ca^{2+} -independent desensitization mechanisms in VSM are involved in the relaxant effect of AMPK in intrarenal arteries.

EDHF, VSM, AMPK, renal interlobar arteries

P2-04

STUDY OF THE EFFECTS OF A MARJORAM EXTRACT (ORIGANUM MAJORANA) ON INFLAMMATION AND VASCULAR FUNCTION IN WISTAR RATS

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Origanum majorana L (marjoram) is an edible herb with high percentage of phenolic acids and flavonoids that exerts antioxidant, antimicrobial and anti-inflammatory activities; however its vascular effects have been poorly studied. Therefore the aim of this study was to analyze the effects of a marjoram extract on vascular reactivity in tail artery segments as well as to analyze the possible beneficial effects or marjoram extract preventing the molecular alterations in aorta segments after an inflammatory challenge. The marjoram extract was obtained by pressurized liquid extraction (PLE) and its components were characterized and quantified by HPLC. The two most abundant compounds in the extract were 6-hidroxiluteolin-7-O-glucoside (40.3 mg/g) and rosmarinic acid (36.2 mg/g). For the vascular studies, 4 month-old male Wistar rats were used. After sacrifice, the tail artery was dissected, cut in 2 mm rings and set in an organ bath to perform vascular reactivity experiments in response to both vasodilator and vasoconstrictive substances in presence/absence of marjoram extract (200 μ g/ml). The aorta also was dissected, cut in 2 mm segments and incubated overnight in the presence/absence of LPS (100 ng/ml) and different concentrations of marjoram extract (20 μ g/ml, 200 μ g/ml and 500 μ g/ml). The release of nitrites to the culture medium was quantified by the Griess method and the gene expression of different markers related to both inflammation and oxidative stress was evaluated by RT-PCR in arterial tissue. The marjoram extract exerted a direct vasodilator effect and a decreased vasoconstrictor action in response to norepinephrine (NE), endothelin-1 (ET-1) or angiotensin II (ANGII) in tail artery segments, although its mechanism of action remains unclear. In aorta segments, the marjoram extract did not modify the mRNA levels of endothelial nitric oxide synthase (eNOS), tumor necrosis factor alpha (TNF α), glutathione reductase (GSR), and NADP oxidase 1 (NOX-1) although it attenuated the LPS-induced-overexpression of inducible nitric oxide synthase (iNOS), interleukin 1 β (IL-1 β), interleukin 6 (IL-6) and interleukin 10 (IL-10) and restored the LPS-induced downregulation of glutathione peroxidase 3 (GPX-3) mRNA levels. Furthermore, incubation of aorta segments with the marjoram extract significantly upregulated the gene expression of superoxide dismutase-1 (SOD-1) and decreased the nitrites concentration in the culture medium. In conclusion, the marjoram extract exerts vasodilator and anti-vasoconstrictor effects and attenuates the arterial damage after an inflammatory challenge. The results derived from this work postulate marjoram extract as a possible treatment for vascular disorders such as hypertension.

Marjoram, cardiovascular system, aorta, inflammation

P2-05

STUDY OF THE EFFECTS OF A MARJORAM EXTRACT (ORIGANUM MAJORANA) ON CARDIAC DAMAGE INDUCED BY CORONARY ISCHEMIA-REPERFUSION IN WISTAR RATS

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Origanum majorana L (marjoram) is an edible herb with high percentage of phenolic acids and flavonoids that exert antioxidant, antimicrobial and anti-inflammatory effects. However, its cardiac effects have been poorly studied. The aim of this study was to analyze the effects of a marjoram extract in the prevention of functional and molecular alterations induced by coronary ischemia-reperfusion (IR) in rats. The marjoram extract was obtained by pressurized liquid extraction (PLE) and its components were characterized and quantified by HPLC. The two most abundant compounds in the extract were 6-hidroxitiluteolin-7-O-glucoside (40.3 mg/g) and rosmarinic acid (36.2 mg/g). For the cardiac studies, 4 month-old male Wistar rats were used. After sacrifice, hearts were subjected to 30 min of ischemia followed by 45 min of reperfusion (IR) in a Langendorff system whereby they were perfused with Krebs solution in the presence/absence of vehicle (70% ethanol) or marjoram extract (200 µg/ml). Control hearts were not subjected to IR. Changes in coronary perfusion pressure, heart contractility (dp/dt) and heart rate were recorded before and after IR. After perfusion, hearts were collected and kept frozen to further analyze the gene expression of different markers related to both inflammation and oxidative stress in cardiac tissue by RT-PCR. The hearts treated with the marjoram extract showed decreased heart rate, a potent vasodilator effect in coronary arteries and increased contractility after IR compared to non-treated hearts. These functional effects were associated with a significant decrease in the gene expression of the NADP oxidase 1 (NOX-1) and with an upregulation in the mRNA levels of antioxidant markers such as superoxide dismutase-1 (SOD-1) and glutathione reductase (GSR). On the contrary treatment with the marjoram extract did not prevent neither the IR-induced-downregulation in endothelial nitric oxide synthase (eNOS) gene expression nor the IR-induced-upregulation in inducible nitric oxide synthase (iNOS) mRNA levels. Likewise the gene expression of pro-inflammatory markers such as tumor necrosis factor alpha (TNFα), interleukin 1β (IL-1β) or interleukin 6 (IL-6) and anti-inflammatory cytokines such as interleukin 10 (IL-10) were not affected by marjoram treatment. In conclusion, marjoram extract attenuates the cardiac damage induced by IR possibly through the activation of antioxidant mechanisms. Thus marjoram extract is a promising functional food that could be used as a potential treatment for cardiac diseases such as myocardial ischemia.

Marjoram, cardiovascular system, heart, ischemia-reperfusion

P2-06

ALTERATIONS IN PROTEIN KINASE C ALPHA AND RHOA/RHO KINASE PATHWAYS, AND L-TYPE CALCIUM CHANNELS INDUCED BY SUBARACHNOID HAEMORRHAGE IN RATS

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Background information: Protein kinase C (PKC), RhoA/Rho-associated kinase (ROCK) pathways and L-type calcium channels (LTCCs) play important roles in sustained arterial contraction. They may participate in pathological situations where vascular smooth muscle cells (VSMCs) are depolarized and arterial contractility is increased. Subarachnoid haemorrhage (SAH) is a cerebrovascular pathology, produced by a rupture of cerebral blood vessels, that can induce cerebral vasospasm and, as a consequence, a high morbidity-mortality in affected patients. **Objective:** To explore whether PKC and RhoA/ROCK pathways and LTCCs could be altered in a SAH rat model.

Methodology: All experiments were conducted in accordance with Spanish R.D. 53/2013 and European Union 2010/63/EU legislation on protection of experimental animals. Using a SAH rat model we explored which pathways involved in arterial contraction could be affected and how this pathology changes them. Animals were divided in 3 experimental groups: control, sham and SAH. Sham and SAH groups were evaluated on days 1, 5, 7 and 9 after surgery. Electrophysiological recordings were performed on days 5, 7 and 9 after surgery, using Circle of Willis VSMCs. Resting potential was measured using the patch-clamp technique in current-clamp configuration. For myograph experiments, basilar arteries were removed from anesthetized animals, cleaned of the adjacent connective tissue, cut in rings and mounted on a small-vessels myograph. Arterial rings reactivity was evaluated, on days 5 and 9 after surgery, in response to high potassium solution using GFX, a PKC inhibitor; fasudil, a ROCK inhibitor; and nifedipine, an LTCCs antagonist. PKCα and RhoA expression was analyzed on days 1, 5, 7 and 9 by western blots using total extracts from cerebral arteries.

Results: Electrophysiological analysis showed that the resting membrane potential of VSMCs isolated from cerebral vessels was depolarised in SAH groups versus sham long time after surgery. Pharmacological experiments performed in a wire myograph system using GFX, a PKCα inhibitor; fasudil, a ROCK inhibitor; and nifedipine, a LTCCs antagonist, indicated that RhoA/ROCK pathway and LTCCs could be chronically affected after SAH. PKCα seemed not be functionally affected after SAH. Western blot analysis showed that PKCα and RhoA expression were both altered after SAH.

Conclusions: SAH could induce alterations in the mechanisms responsible for sustained contractions in cerebral arteries from the affected areas. Our results suggest that several signalling pathways involved in maintained contraction may be modified in our rat SAH model.

PKC alpha, RhoA, Subarachnoid haemorrhage

P2-07

DYNAMICS OF PHASE SINGULARITIES DURING VENTRICULAR FIBRILLATION IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME

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Background: It has been reported that patients with metabolic syndrome (MetS) have a higher incidence of arrhythmias that are non-related to ischaemic events, suggesting a pro-arrhythmic structural and/or electrical

remodeling, but the underlying mechanisms are not completely understood. **Objective:** To investigate the electrophysiological mechanisms underlying VF an experimental model of diet-induced MetS by means of the study of the spectral and phase-domain characteristics of the arrhythmia. **Methods:** Male NZW rabbits were randomly assigned to a control (n=12) or a MetS group (n=13), fed during 28 weeks with high-fat (10% hydrogenated coconut oil and 5% lard), high-sucrose (15% dissolved in water) diet. After 28 weeks, their hearts were isolated and perfused in a Langendorff system and epicardial optical mapping was performed using two synchronized EMCCD cameras focused on the left (LV) and right (RV) ventricles (field of view: 128 x 128 pixels; sampling frequency: 330 frames/second). We used the electromechanical uncoupler blebbistatin (7.5 μ M) and the potentiometric dye di-4-ANBDQPPQ. VF was induced by pacing at increasing frequency (intensity= 3 x diastolic threshold) and, once triggered, 10 s recordings were made each minute, during 6 minutes (without interrupting perfusion). We analyzed VF characteristics by means of spectral dominant frequency (DF), phase-domain normalized singularity point density (SPd), and SPd at the 95% fastest activated domain (SPd95). A factorial ANOVA was used for statistical analysis (p<0.05). **Results:** Spectral analysis showed that DF increased in the RV of MetS animals (10.3 \pm 1.0 vs. 11.4 \pm 1.1; p<0.05), even though we did not find differences in the LV between both groups. After phase singularity analysis, we did not find differences in SPd and SPd95 when comparisons were made between control and MetS groups, or within each group when comparing RV vs. LV. No correlation was found between DF and any of the phase singularity analysis parameters. **Conclusion:** Frequency of activation during VF increased in the RV of animals with MetS, whereas there was no difference in the LV. Since phase singularities were not altered in the RV of MetS animals, in our experimental model, reentry does not seem to be the only electrophysiological mechanism underlying the increased frequency of activation during VF. **Acknowledgements:** This work was supported by UV-INV-PRECOMP14-206372 (Universitat de València), GV2015-062 (Generalitat Valenciana), PI15/01408 and CIBERCV CB16/11/0486 ISCIII and FEDER funds.

Metabolic syndrome, ventricular fibrillation, electrophysiology

P2-08

ALTERATIONS IN HEART RATE VARIABILITY AND ITS CIRCADIAN REGULATION IN AN EXPERIMENTAL MODEL OF DIET-INDUCED METABOLIC SYNDROME

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Background: Metabolic syndrome (MetS) is a clustering of metabolic and cardiovascular risk factors: high blood pressure, dyslipidemia, elevated fasting glucose, and central adiposity. MetS has been linked with a higher prevalence of cardiovascular mortality, including sudden cardiac death (SCD), but the mechanisms are not well understood. One possible mechanism underlying the increased arrhythmogenesis may be an abnormal modulation of autonomic activity, quantified analyzing heart rate variability (HRV).

Objective: To investigate the modifications that MetS produces in long-term HRV and its possible effects on the circadian rhythms. **Methods:** Male NZW rabbits were randomly assigned to control (n=7) or MetS group (n=7), fed during 28 weeks with high-fat, high-sucrose diet. At week 28, after anesthesia, two electrodes were sutured in the animal chest and connected to a recording device (Emotion Faros 180, Mega Electronics®). Once the animal recovered consciousness, 24 h recordings

were made with a sampling rate of 1 kHz. We analyzed 1 hour recordings from 12:00 to 1:00 AM (day, D) and 12:00 to 1:00 PM (night, N). Standard HRV parameters were determined: 1) Time domain: RR, HR, SDNN, triangular index (Ti), RMSSD and TINN; 2) Frequency domain: very low frequency (VLF), low frequency (LF), high frequency (HF), and LF/HF; 3) Non-linear analysis: Poincaré (SD1, SD2) and different entropy analyses. Analysis of variance (MANOVA) was used for statistical analysis (p<0.05).

Results: RR decreased in MetS animals during both D and N when compared to controls (D: 244 \pm 20 vs. 220 \pm 15 ms; N: 245 \pm 18 vs. 207 \pm 15 ms; p<0.05), reflecting an increased heart rate, but no differences were found within control and MetS group comparing D vs. N. The same trend was observed for TINN (D: 188 \pm 37 vs. 147 \pm 39 ms; N: 167 \pm 22 vs. 130 \pm 47 ms; p<0.05) and the triangular index (D: 6.9 \pm 1.4 vs. 4.6 \pm 1.2 a.u.; N: 6.9 \pm 1.9 vs. 4.2 \pm 1.7 a.u.; p<0.05). No differences were found in the rest of time domain parameters. With respect to the frequency domain, AR spectrum analysis did not find any change in VLF, LF, HF and LF/HF. Nonlinear analysis showed a decreased SD2 in MetS animals during N (32.5 \pm 7.3 vs. 19.4 \pm 8.4 ms; p<0.05).

Conclusion: MetS produced a decrease in RR interval duration, triangular index and TINN, suggesting an increased sympathetic activity during day and night. Those changes were not reflected in LF modifications, as we had expected. SD2 component of Poincaré analysis also decreased during night which also suggests a non-reciprocal decreased parasympathetic activity.

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Metabolic syndrome, heart rate variability

P2-09

TIME AND FREQUENCY DOMAIN ANALYSIS OF SHORT-TERM HEART RATE VARIABILITY IN AN EXPERIMENTAL MODEL OF DIET-INDUCED METABOLIC SYNDROME

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Background: Metabolic syndrome (MetS) is defined as at least three of the following conditions: central obesity, elevated triglycerides, decreased high-density lipoproteins, systemic hypertension, and glucose intolerance. MetS is linked with a high prevalence of cardiovascular disease and sudden cardiac death, events that may be a consequence of changes related to the structure, function and control of the cardiovascular system. One underlying mechanism could be the alteration of sinus node automaticity caused by problems in the neural control which, in turn, can trigger cardiac arrhythmias. Heart rate variability (HRV) analysis is a non-invasive tool useful for evaluating alterations in neural control of the heart. **Objective:** To examine the changes in short-term HRV using time- and frequency-domain analysis in an experimental model of diet-induced MetS, and its evolution during the period of induction.

Methods: Male NZW rabbits were randomly assigned to control (n=10) or MetS group (n=13), fed with high-fat, high sucrose diet during 28 weeks. After anesthesia, 15 min ECG recordings were performed (lead I), before diet administration and at weeks 14 and 28. We analyzed short RR time series and quantified the standard parameters in time and frequency domains: 1) Time domain: RR, HR, SDNN, triangular index (Ti), RMSSD and TINN; 2) Frequency domain: very low (VLF), low (LF),

high frequency (HF), and LF/HF. Multivariate analysis of variance (MANOVA) was used for statistical analysis ($p < 0.05$). **Results:** Frequency domain analysis of HRV showed an increase in the HF component in MetS animals at week 28 (1.4 ± 1.4 vs. 5.9 ± 5.5 %; $p < 0.05$). In addition, the LF component increased in MetS animals at weeks 14 and 28 when compared to controls (week 14: 3.6 ± 2.6 % vs. 12.7 ± 10.7 %; week 28: 2.2 ± 2.1 % vs. 14.4 ± 10.7 %; $p < 0.05$). No changes were observed in the rest of frequency-domain components, or when comparisons were made within groups between pre-diet, week 14 and week 28.

Conclusion: Both parasympathetic and sympathetic tone increased by feeding a high-fat, high-sucrose diet, without changes in sympatho-vagal balance. Short-term HRV analysis does not seem sensitive enough to detect changes during MetS induction, thus further analyses using long-term HRV are advised in order to identify potential early markers of this syndrome using HRV.

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Metabolic syndrome, heart rate variability

P2-10

MODIFICATIONS OF SHORT-TERM INTRINSIC PACEMAKER VARIABILITY IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME. A STUDY ON ISOLATED RABBIT HEART

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Background: Metabolic syndrome (MetS) describes an association between diabetes, hypertension, obesity and dyslipidemia. It has been linked with a higher prevalence of cardiovascular disease, arrhythmogenesis and sudden cardiac death. Indeed, one of the underlying mechanism to explain those events could be an altered automaticity, which would reflect modifications of the sinus node activity. These phenomena can be evaluated analyzing the components of heart rate variability (HRV).

Objective: To examine the modifications of sinus node variability in an isolated heart model of diet-induced MetS.

Methods: Male NZW rabbits were randomly assigned to control ($n=10$) or MetS group ($n=12$), fed with high-fat (10% coconut oil and 5% lard) and high sucrose (15% dissolved in water) diet during 28 weeks. After euthanasia their hearts were isolated in a Langendorff system and, after 15 minutes of stabilization, we recorded 15 minutes of spontaneous activity. Short RR time series (600-900 s) were analyzed and standard HRV parameters were determined: 1) Time domain: RR, HR, SDNN, triangular index (Ti), RMSSD and TINN; 2) Frequency domain: very low frequency (VLF), low frequency (LF), high frequency (HF), and LF-HF ratio, using autoregressive model spectrum analysis; 3) Non-linear analysis: Poincaré (SD1, SD2) and different entropy analyses. Unpaired t-test or Mann Whitney U test were used when necessary ($p < 0.05$).

Results: In the frequency domain, we did find an increase in the LF component of HRV in MetS animals (12.6 ± 15.5 vs. 29.9 ± 18.3 %; $p < 0.05$) and LF/HF ratio (1.18 ± 0.7 vs. 2.8 ± 1.5 %; $p < 0.05$), but the rest of the frequency domain parameters (VLF and HF) remained unchanged. With respect to nonlinear analysis, approximate entropy (ApEn; 0.35 ± 0.27 vs. 0.07 ± 0.05

a.u.; $p < 0.05$) and the minimum of multiscale entropy (MSEmin; 0.23 ± 0.15 vs. 0.11 ± 0.07 a.u.; $p < 0.05$) decreased in MetS group. No differences were found in any of the standard time domain parameters (RR, STDRR, RMSD, NN50, RR triangular index and TINN).

Conclusion: The increase in LF component, although controversial, has been associated to impaired baroreflex (stretch-sensitive mechanoreceptors) and augmented sympathetic activity. Even though the isolated heart is not submitted to baroreflex, extrinsic nervous or humoral control, we cannot exclude participation of the intrinsic cardiac nervous system which, although it has been shown to be inactive after acute decentralization in healthy non-working hearts, could play a role in the observed modifications. The decreased entropy in MetS animals reflects a higher regularity and loss of complexity of RR interval dynamics, which has been related to pathological states.

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Metabolic syndrome, intrinsic heart rate variability

P2-11

OVEREXPRESSION OF NADPH OXIDASE AND OXIDATIVE IMBALANCE IN THE EYE OF HYPERTENSIVE RATS

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Arterial hypertension (AH) is associated with enhanced production of reactive oxygen species (ROS) that participate in the initiation, maintenance and progression of hypertensive disease via different molecular mechanisms, including inflammatory and fibrotic processes. Specifically, it has been suggested that AH is involved in the development of ocular diseases such as glaucoma, retinopathies, cataracts and choroidopathies. Despite the evidences linking AH with alterations in ocular structure/function, the mechanisms involved in these regard are not well known. Considering that the enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, is the most important source of ROS in hypertension-related organ damage, the aim of this study was to assess the role of ocular NADPH oxidase in the hypertensive context. To this purpose, NADPH oxidase activity, as well as protein/mRNA expression of: i) NADPH oxidase isoforms (NOX1, NOX2, NOX4) and subunits (p22phox, p47phox); ii) nitric oxide synthase isoforms (endothelial, eNOS and inducible, iNOS); iii) antioxidant enzymes (namely, glutathione peroxidase, GSH-Px, glutathione reductase, GSH-Red, and superoxide dismutase, SOD); and iv) nitrotyrosine, were measured in eyeball homogenates from normotensive (control) and hypertensive Wistar rats treated with N ω -nitro-L-arginine methyl ester (L-NAME). The eyes were enucleated and homogenized at 4°C with a Potter-Elvehjem tissue grinder. Then, the activity of intracellular NADPH oxidase was measured by lucigenin-enhanced chemiluminescence in a tube luminometer. Protein and mRNA expression were measured by Western blotting and real time PCR, respectively. Our results showed an increase in NADPH oxidase activity and subsequent release of superoxide anion in ocular tissue from hypertensive animals, which was accompanied by upregulation of the main isoforms of this enzyme (NOX). Furthermore, L-NAME-induced hypertension resulted in altered expression of NOS and antioxidants enzymes. In conclusion, • Arterial hypertension produces an increase in local oxidative stress in the eyeball that might be related with those

pathophysiology mechanisms responsible for the development of ocular diseases. • The oxidative status is altered in L-NAME-treated hypertensive rats, which highlights the relationship between arterial hypertension, oxidative stress and subsequent development of ocular pathologies.

Arterial-hypertension, Eyeball, L-NAME, NADPH-oxidase, Nitric-oxide, Oxidative-stress

P2-12

CLUSTERING OF MOLECULES RELATED TO ARTERIOSCLEROTIC CAROTID DAMAGE IN PATIENTS WITH CRITERIA OF METABOLIC SYNDROME

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Introduction: Metabolic syndrome (MetS) includes subjects with different risk factors for developing cardiovascular disease (CVD). However, with the current criteria a heterogeneous group of individuals is diagnosed of SM, whose clinical and prognostic features are very different, and not all have the same risk of developing CVD. **Objective:** The aim of this study is to identify parameters that will help us better stratification of the concrete risk of developing CVD.

Methods: 78 patients with MetS according to NCEP ATP III criteria were included. Plasma concentrations of adipocytokines (IL-6, TNF- α , IL-1b, IL-8, IL-18, MCP-1), adipocyte-derived hormone (adipsin, leptin, visfatin, adiponectin, omentin, resistin), ghrelin and cellular adhesion molecules (VEGF, PAI-1, ICAM-1, VCAM-1) were determined using a customized multiplex assay (Luminex Assay) according to manufacturer's instructions. Clinical and biochemical parameters data related with CVD risk factors and arteriosclerotic carotid damage (intima-media thickness [IMT] > 0.9 mm) were collected.

Results: A hierarchical cluster analysis was performed to shed light on plasma molecules with a potential diagnostic role in CVD. The analysis revealed the presence of six main clusters (cluster I to 6) within MetS patients and two clusters of cytokines. The first cytokine cluster (cluster I) encompassed IL-6, TNF- α , VEGF, adipsin, IL-1b, IL-8, leptin, PAI-1, IL-18 and visfatin, while the second one (cluster II) consisted on ghrelin, adiponectin, omentin, MCP-1, resistin, ICAM-1 and VCAM-1. Patient cluster 3 included the highest percentage of patients undergoing IMT, and they showed high concentration of group II cytokines while group I was reduced. On the other hand, patient cluster 1 included the lowest proportion of patients with GIM, and group I cytokine concentration was, however increased.

Conclusions: Plasma molecule profiling may provide an insight in MetS patient prognosis and will improve the current follow up.

Metabolic syndrome, cardiovascular disease, cytokines

P2-13

ROLE OF STORE-OPERATED AND L-TYPE CALCIUM CHANNELS IN CORONARY ARTERY HYPERCONTRACTION INDUCED BY ENDOTHELIN-1 AFTER ISCHEMIA AND REPERFUSION

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Introduction: Ischemia-Reperfusion (I/R) injury triggers a variety of cellular dysregulation, including alteration of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and coronary hypercontraction. Here, we evaluated the role of Store-Operated Ca^{2+} Channels (SOCCs) and L-type Ca^{2+} channels (LTCCs) in coronary artery vasoconstriction originated by endothelin-1 (ET-1) under I/R.

Methods: Contractility and changes in the $[Ca^{2+}]_i$ under simulated I/R protocol were studied using left anterior descending coronary arterial rings and isolated smooth muscle cells from Wistar rats.

Results: We observed that coronary vascular tone under I/R was not altered, however, vasoconstriction and the increase of $[Ca^{2+}]_i$ evoked by high-KCl-induced depolarization was significantly attenuated under I/R. Similarly, intracellular $[Ca^{2+}]$ stores was also decreased during I/R. In addition, coronary arteries exposed to I/R and treated with ET-1 showed small and transient increase of $[Ca^{2+}]_i$ and coronary vascular tone, leading to vasoconstriction during reperfusion. ET-1-induced vasoconstriction and $[Ca^{2+}]_i$ rise during reperfusion were sensitive to inhibitors of SOCCs and LTCCs. Finally, using in situ Proximity Ligation Assay we observed that ET-1 enhanced significantly the colocalization of Orail1, subunit of SOCCs, with L-type $CaV1.2$ channel under I/R.

Conclusions: Our data confirm that ET-1-induced contraction of coronary artery after a process of ischemia-reperfusion involve the co-activation of LTCCs and SOCCs.

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Coronary-Artery, Endothelin-1, Ischemia-Reperfusion, Orail-TRPC1, SOCC, LTCC

P2-14

STUDY OF THE VASCULAR DAMAGE IN AORTAS FROM SUNITINIB TREATED RATS

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Aims: Sunitinib (Su) is a tyrosine kinase receptor inhibitor with antiangiogenic and antineoplastic properties. The purpose of this study was to measure the endothelial function and vascular remodeling, as well to analyze the NADPH oxidase system, in aortas obtained from Su-treated rats, in order to investigate the mechanisms involved in the increase of the blood pressure observed after treatment with this drug.

Materials and methods: Male Wistar rats were randomly assigned into 2 groups: i) control group (free access to tap water); and ii) rats treated with 25 mg Su/kg body weight/day. During the 2 weeks of treatment, blood pressure and heart rate was continuously monitored. Upon completion of treatment, rats were humanely killed and the thoracic aorta was immediately dissected. Aortic homogenates were prepared to study the activity and expression of the enzyme NADPH oxidase, and aortic rings were used for vascular reactivity experiments and histological analysis.

Results: As expected, Su-treatment resulted in a significant elevation of blood pressure parameters. The vascular function study showed no differences among the groups in terms of the vasoconstrictor response to the adrenergic agonist, phenylephrine. On the other hand, a decrease in endothelium-dependent vasodilation (in response to acetylcholine) was observed in aortic rings from Su-treated rats with respect to the control group. The remaining endothelium-dependent vasodilator response was equal in both groups after pre-incubation with the nitric oxide synthase inhibitor, L-NAME. Endothelium-independent relaxation in response to sodium nitroprusside was unaltered by Su treatment. In addition, histological analysis demonstrated the presence of vascular remodeling, with thickening and increased cross sectional area of tunica media, in Su-treated rats. Similarly, a significant increase in the activity of the pro-oxidant enzyme NADPH oxidase was observed in the aortas of Su-treated rats; a result that correlates with enhanced gene expression of NADPH oxidase isoforms NOX1, NOX2 and NOX4.

Conclusions: A daily administration of sunitinib for two weeks seems to be sufficient for the development of hypertension, which might be due to endothelial dysfunction and vascular remodeling processes induced by the drug; such effects of sunitinib might be secondary, at least in part, to excessive activation of the NADPH oxidase system.

Sunitinib, Arterial-hypertension, Endothelial-dysfunction

P2-15

IMMUNE CELL SUBSET ANALYSIS AND CYTOKINE PROFILING IN PATIENTS WITH CHRONIC SILICOSIS CAUSED BY ARTIFICIAL QUARTZ AGGLOMERATES

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Silicosis produced by Artificial Quartz Agglomerates (AQA) evolves more aggressively than the classical form of miners. This entity is emerging worldwide and a significant group of cases has been detected in the province of Cádiz (Spain) in recent years. The role of the cellular immune response in the pathogenesis of silicosis by AQA has not been previously studied. Then, 60 patients diagnosed with silicosis by AQA and 18 healthy controls were studied to analyse cell populations and the cytokines present in peripheral blood and compared between them. The aim of the study was to obtain a correlation between that analysis and the multiple clinical parameters with the aim of having a prognostic value or treatment guideline for patient evolution. The blood cells populations and the cytokine levels were quantified by flow cytometry and multiplex ELISA analysis, respectively. In general, no differences were found in the total number of leukocytes or granulocytes. However, a significant increase in monocytes cell number and a clear lymphocytopenia were observed in the blood from patients compared to healthy controls. Almost all the lymphocyte subsets studied - B lymphocytes, T lymphocytes and NK cells ('Natural Killers') - were decreased compared to healthy controls. Particularly, a significant decrease in the total cell number was observed in the following subsets: memory B lymphocytes; T-helper lymphocytes, naïve and memory T-lymphocytes, T-regulatory lymphocytes and CD56Bright NK cell subpopulations. However, there was a significant increase in the TH1 and TH17 subsets as well as in plasma cells in patients. As a conclusion, the alterations in blood cell populations and cytokine profiling could reflect different states of inflammatory and fibrotic activity in these

patients and it could be used as indicator of the disease progression as well as for type/regimen of treatment.

Immune cells, cytokines, Silicosis, Human

P2-16

SHORT-TERM TREATMENT WITH ESMOLOL CONFERS CARDIOPROTECTION IN SPONTANEOUSLY HYPERTENSIVE RATS VIA INHIBITION AKT/NF- κ B AND NFATc4

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Background: Our group has previously demonstrated that short-term treatment with esmolol reduces left ventricular hypertrophy (LVH) in spontaneously hypertensive rats (SHRs) ⁽¹⁾. However, molecular mechanisms of this effect have not been analyzed to date. The present study is aimed to assess whether short-term treatment with esmolol reverses LVH in aged SHR by down-regulation of Akt/NF- κ B and NFATc4 activity.

Methods: Fourteen-month-old male SHRs were treated intravenously with saline as vehicle (SHR) or esmolol (SHR-E) (300 microg/kg/min). Age-matched vehicle-treated male Wistar-Kyoto (WKY) rats served as controls. After 48 hours of treatment, the hearts were harvested and left ventricular tissue separated and processed for Western blot analysis to determine the levels of Akt, NF- κ B, NFATc4, Creb1, Serca2a, Erk1/2, Sapk/Jnk. The parameters were compared using single-factor analysis of variance and a post hoc Bonferroni correction was applied. All procedures were approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón, Madrid, Spain;

Results: Esmolol had the following effects on LVH: a) It reversed the levels of p-NFATc4 in SHR rats to the control WKY levels, but did not modify the expression of p-Creb1 and Serca2a in SHR rats; b) It also reversed the levels of p-Akt and p-NF- κ B in SHR rats to the phospho-levels of these proteins in WKY rats without modifying p-Erk1/2 or p-Sapk/Jnk.

Conclusion: Short-term treatment with esmolol reverses LVH in aged SHR rat by down-regulation of Akt/NF- κ B and NFATc4 activity.

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References: ⁽¹⁾ Quintana-Villamandos B et al. Hypertension Res 2013;36:408-413.

Esmolol, Ventricular Hypertrophy, NFATc4

P2-17

MODIFICATIONS OF DYNAMIC RESTITUTION DURING INDUCTION OF VENTRICULAR FIBRILLATION IN A MODEL OF METABOLIC SYNDROME

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Introduction: Metabolic syndrome (MetS) involves a cluster of cardiovascular and metabolic conditions occurring simultaneously that have been linked to risk of cardiovascular disease, stroke and sudden cardiac death (SCD). There is increasing evidence of a progressive structural and/or functional remodeling that may impact cardiac electrophysiology, arrhythmogenesis and SCD events in obese and diabetic patients. However, the underlying mechanisms are poorly understood. Restitution properties of electrophysiological adaptation are widely considered to play a key role into the initiation and perpetuation of ventricular fibrillation (VF). Our aim was to explore dynamic restitution properties of action potential duration (APD) and oscillatory vulnerability windows before VF induction in an experimental model of MetS.

Methods: Fourteen male NZW rabbits were assigned to a control (Ctrl, n=7) or MetS group (n=7), fed during 28 weeks with high-fat, high-sucrose diet. After week 28th, hearts were isolated and perfused into Langendorff preparations. Pacing and recording electrodes were placed into the LV, and pacing stimulation under increasing frequency induced VF. Optical mapping (OM) was performed using a synchronized dual-chamber setup with a sampling frequency of 330frames/s. We used the electromechanical uncoupler blebbistatin and the potentiometric dye di-4-ANBDQPQ. Continuous recording of unipolar ECG, and OM movies at 350, 400, 500, 550, 600, 650 BPM were acquired without interrupting perfusion. We analyzed APD at 90% of repolarization restitution curves, their slopes and the presence of oscillatory events during restitution. A mixed-model ANOVA and unpaired t-tests were used for statistical analyses ($p < 0.05$).

Results: Progressive APD shortening was greater in MetS animals in the RV at slower pacing frequencies while no differences were found in the LV, as observed during fixed-frequency stimulation protocols. At faster rates, before induction, a trend was found in the maximum restitution slope, being steeper in both ventricles under MetS (i.e. 0.94 ± 0.17 vs 0.82 ± 0.06 in RV; $p < 0.05$). APD oscillations were 8.2% higher under MetS in the RV, which could be predicted by changes in the maximum restitution slope and could partially explain the arrhythmogenic mechanism under MetS. We also observed the presence of oscillatory events during restitution. However, no differences were found into the minimum frequency for VF induction in the LV (7.91 ± 3.27 vs 8.04 ± 0.88).

Conclusion: Simultaneous dual-chamber mapping of epicardium in an experimental model of MetS showed modifications in electrical remodeling suggesting an implication of frequency-dependent adaptation at faster rates into the mechanisms of VF under MetS.

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Metabolic syndrome, Ventricular Fibrillation, Electrophysiology

P2-18

DRONEDARONE IMPROVES GLOBAL ANTIOXIDANT STATUS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objectives: We previously demonstrated that dronedarone reverses left ventricular hypertrophy (LVH) in an experimental model of arterial

hypertension.⁽¹⁾ One of the mechanisms involved could be a reduction in oxidative stress. Therefore, a global oxidative stress index (Oxy-Score) was developed using spontaneously hypertensive rats (SHR).

Materials and Methods: adult male SHR rats were randomly divided into dronedarone group (SHR-D, n=9) and placebo group (SHR, n=9). Wistar Kyoto rats were used as normotensive controls (WKY, n=9). After 14 days of treatment we analyzed the following plasma biomarkers of oxidative status: protein carbonyls, thiols, total antioxidant capacity, superoxide anion scavenging activity and reduced glutathione. We calculated a global score using the statistical methodology previously described⁽²⁾. Groups were compared using single-factor analysis of variance and a post hoc Bonferroni correction was applied. All data was expressed as mean \pm SEM. $P < 0.05$ was considered significant. All procedures were approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón, Madrid, Spain;

Results: we didn't found significant statistical differences among the three groups when studying individual parameters. But, when we studied the Oxy-Score, we saw interesting differences between SHR and SHR-D group. The SHR Oxy-Score was negative, meaning a predominance of the oxidative damage. Whereas the SHR-D Oxy-Score resulted positive, indicating a predominance of the antioxidant capacity versus the oxidative damage ($p < 0.001$).

Conclusions: Dronedarone improves the global antioxidant status in SHR. This could be a mechanism by which the drug would exercise its positive effect in the regression of LVH.

Acknowledgements: This work was supported by a grant from FIS13/01261 PI16/02069 and Fondos FEDER, Spain.

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Dronedarone, oxidative stress, arterial hypertension

P2-19

DOSE-RESPONSE CALCIUM TRANSIENT MODIFICATIONS BY RANOLAZINE ON RESTITUTION AND SPONTANEOUS RE-ENTRANT ACTIVITY USING A CUSTOM CARDIAC MONOLAYER OPTICAL MAPPING SETUP

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Introduction: Despite substantial advances in catheter ablation, antiarrhythmic drugs are the mainstay of treatment in atrial fibrillation (AF). Fast activation during fibrillation promote ion channel instability and impaired calcium handling, among other factors. Ranolazine (RZN) is atrial selective, inherently safe and has shown to terminate cholinergic and stretch models of AF. Our purpose was to evaluate the effect of RZN on calcium (Ca^{2+}) dynamics during pacing and spontaneously arising re-entry.

Methods: An optical mapping (OM) system for cardiac monolayers was setup including units for controlling illumination, temperature, perfusion and electrical stimulation using Matlab and Labview. We prepared HL1 atrial-like cell monolayers in confluent cultures using standard 35mm dishes (n=11). High-resolution Rhod2AM (Ca^{2+})-OM was performed at 1000fps. Spontaneous re-entrant activity was observed in all cultures. We

also monitored every 2 min., during 10 min periods, the effect of 5uM, 10uM and 20 uM RZN. Spectral properties, entropy, pattern complexity and normalized density of singularity points (SPd, SP/cm²/s) were analysed. Additionally, controlled stimulation at 500, 800, 1200 and 1500ms was done. Calcium transient durations (CaTD) characteristics and conduction velocities were quantified. A mixed-model ANOVA and unpaired t-tests were used for statistical analyses (p<0.05).

Results: We recorded fluorescence signal-to-noise ratios of 29±8dB. Spontaneously arising re-entry led to various degrees of complexity where the initial number of wavelets correlated well with days-in-culture. Maximum and mean dominant frequency were reduced in presence of RZN from 5-to-10-to-20uM (2.14±0.63, vs 1.82±0.41, vs 1.24±0.23 vs 0.64±0.44; Ctrl vs 5uM, vs 10uM, vs 20uM, p<0.05), with regularized activation, as shown by higher spectral organization and lesser signal entropy. Activity stopped in 7 out of 11 dishes at higher concentrations. Complexity was progressively reduced shown by re-entrant waves with larger re-entrant cores and lesser curvature wave-fronts. After RZN treatment, SPd was dramatically reduced (4.21±0.95 vs 0.42±0.19, control vs 20uM, p<0.05). During stimulation CaTD was unaffected at slower rates. CaT showed regularized activation-recovery with no oscillatory events under RZN during neither fast nor slow activation. Conduction velocity was non-significantly reduced. Likelihood of either mechanical or electrical induction was diminished by 31% and 16%, respectively. **Conclusions:** We validated a cardiac monolayer system for the assessment of drug cardiotoxicity and pro-arrhythmia in-vitro. RZN slowed-down and reduced complexity of spontaneously arising calcium waves reducing re-inducibility of fibrillatory conduction.

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Cellular electrophysiology, Optical Mapping, Atrial Fibrillation

P2-20

DIFFERENTIAL CONTRIBUTION OF NOX1, NOX2 AND NOX4 TO RENAL VASCULAR OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN OBESITY

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Oxidative stress and the associated endothelial dysfunction are key pathogenic factors underlying the vascular complications of metabolic disease including diabetic nephropathy (Prieto et al., *Curr Vasc Pharmacol* 12:412-426, 2014 and Thompson et al., *Circ Res* 121:502-511, 2017). NADPH oxidase has been found to be a major source of ROS generation, oxidative stress and renal injury mostly under pathological conditions such as diabetic nephropathy and chronic kidney disease (Ratliff et al., *Antioxid Redox Signal* 25:119-146, 2016 and Sharma, *Antioxid Redox Signal* 225: 208-216, 2016). Therefore, the present study was designed to investigate whether NADPH oxidase may be involved in the renal vascular oxidative stress and to assess their role in the endothelium dysfunction of rat intrarenal arteries from lean Zucker rats (LZR) and obese Zucker rats (OZR). Renal interlobar arteries isolated from the kidney of LZR and OZR rats, were mounted in microvascular myographs to assess function. Superoxide and H₂O₂ production was measured by chemiluminescence and Amplex Red fluorescence and antioxidant enzymes were detected by Western blotting and by double immunolabeling along with eNOS. Under conditions of cyclooxygenase and NO synthase blockade,

endothelium-dependent relaxations were enhanced in OZR and blunted by catalase in both animals and by the Nox2 and the Nox1/4 inhibitors gp91ds-tat, GKT13690, but not by the Nox1 inhibitor NoxA1ds in LZR, this inhibitory effect being reduced in obese rats. Nox4 protein was expressed in the endothelium of renal arterioles and glomeruli colocalized with eNOS, levels of Nox4 being lesser in arteries of OZR than in lean rats. In contrast, Nox2 were up-regulated in renal arteries from OZR. In addition, antioxidant enzymes in vascular tissue including catalase and both cytosolic and mitochondrial SOD were up-regulated in obese rats. Both basal and stimulated superoxide production were increased in arteries of OZR and reduced by apocynin, Nox1, Nox2 and Nox4 inhibitors, in contrast to basal and stimulated vascular H₂O₂ production that was reduced in OZR. These results demonstrate Nox1 and Nox2-derived oxidative stress and renal endothelial dysfunction in obesity, along with an upregulation of antioxidant enzymatic defenses associated to decreased production of Nox-derived H₂O₂ that was regulated with a reduced expression of Nox4, as a protective role in kidney vasculature in obesity.

Endothelial dysfunction, Nox1, Nox4, Nox2

P2-21

CAROB SUPPLEMENTATION REDUCES ENDOTHELIAL DYSFUNCTION IN OBESE MICE: EARLY DETECTION BY LASER SPECKLE

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Obesity is a multifactorial disease that can be characterized as a state of low-grade chronic that in many cases is associated with insulin resistance and an increased risk of suffering cardiovascular diseases. Endothelial dysfunction is a common alteration in several cardiovascular diseases associated to obesity and is produced by increased oxidative stress and a vascular pro-inflammatory state. Anti-obesity drugs can reduce, at least in part, some of the cardio metabolic alterations associated to obesity but some of these drugs present serious side effects that can affect the nervous and cardiovascular systems, among others. For this reason there is a general concern in finding new compounds, with a natural origin and less side effects. CSAT+ is proprietary blend that is composed by carob (*Ceratonia siliqua* L.) gum (Galactomannan) and pods extracts enriched in different groups of antioxidant polar phenols, chiefly gallic acid and derivatives. The aim of this work was to analyze if supplementation with CSAT+, a natural carob extract in the diet, prevents obesity-induced endothelial dysfunction in obese mice. For that purpose three experimental groups of C57BL/6J mice were used: Mice fed a standard chow (Controls), mice fed a high fat/high sucrose diet (HFHS) and mice fed a HFHS diet supplemented with CSAT+ (4.8%) for twelve weeks (HFHS+CSAT+). In order to assess endothelial function, quantification of reactive hyperemia was performed six and twelve weeks after the initiation of the treatment. Mice were anesthetized with a continuous flow of isoflurane in 100% oxygen. Cutaneous blood perfusion was recorded using a Laser Speckle Contrast imaging system and the leg blood perfusion after 3 minutes occlusion through tourniquet was studied. Hyperemia percentage was calculated as: hyperemia peak - pre-occlusion value/pre-occlusion value. The time to peak was the time in seconds between the occlusion release and the hyperemia peak. The hyperemia duration corresponds to the time between occlusion release and the return to the pre-occlusion perfusion value. The repayment / debt ratio was the ratio of the AUC

(Area Under Curve) of the post-occlusive hyperemia and the AUC of perfusion debt during vascular occlusion. No significant changes were found between experimental groups six weeks after the initiation of the treatment. However, in week 12, HFD mice showed decreased hyperemic response compared to controls, and this effect was attenuated by CSAT+ supplementation. In conclusion, CSAT+ supplementation attenuates obesity-induced endothelial dysfunction and these changes can be early detected by a non-invasive technique like the laser speckle.

Endothelial dysfunction, laser speckle, carob supplementation

P2-22

RETINAL RESCUE CORRELATES WITH ROBUSTNESS OF CIRCADIAN LOCOMOTOR ACTIVITY RHYTHMS IN RETINAL DYSTROPHIC RATS TREATED WITH THE CANNABINOID HU210

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The Incidence of circadian rhythm disorders is related to ocular pathologies and blindness. This study aims to examine whether morphologic and functional rescue of retinal degeneration is associated with more robust circadian locomotor activity rhythms in retinal dystrophic rats. Transgenic P23H rats were administered with the synthetic cannabinoid HU210 (100 µg/kg, i.p.) or vehicle three times a week from P24 to P90. Normal Sprague-Dawley rats were used as a healthy control group. The animals were handled according to current regulations for the use of laboratory animals (European Directive 2010/63/UE). At the end of the experimental period, locomotor activity was recorded, the retinal function was evaluated by electroretinography, and the morphology of the retinas was assessed by vertical retinal cryostat sections stained with immunohistochemistry techniques. The ERG a- and b-wave amplitudes and photoreceptor cell number were more deteriorated in vehicle-administered P23H rats than in P23H rats treated with HU210. Vehicle-administered P23H rats also showed weaker circadian locomotor activity rhythms than that observed in HU210-treated P23H rats, the latter showing higher values for mesor, amplitude, acrophase, percentage of variance and non-parametric variables. A positive linear correlation was found between retinal values and circadian parameters of locomotor activity in P23H rats. The results provide evidence for a positive correlation between rescue of retinal structure and function and improvement of circadian rhythmicity.

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Retinal degeneration, P23H, HU210, circadian rhythms

P2-23

CHRONOTYPE, SLEEP QUALITY AND COGNITIVE FUNCTION OF SENIOR UNIVERSITY STUDENTS

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Chronotype is the expression of circadian rhythmicity in an individual and can differ among individuals. Horne and Östberg called attention to

this variability in humans, referring to early risers as “morning types” and to late risers as “evening types”. These chronotypes differ from each other in the timing of physiological variables. Chronotype is an important moderator of sleep schedules and daytime functioning. Characteristics of sleep quality, chronotype and academic performance of young students have been studied. However, there is not data that evaluated the association between these variables among senior university students. The University of Oviedo has developed an education programme aimed at people over 50, the University of Oviedo’s Programme for Mature Students (PUMUO), which consists of a specific offer including course matter of interest to this sector of society. The aim of the present study was to examine the sleep quality and chronotype, as well as their possible association with cognitive function, of the PUMUO students. This study was carried out in accordance with the Declaration of Helsinki. Participants were informed of the aim of the study and the instruments that would be used. A total of 62 PUMUO students (39 females and 23 males; 64.5 ± 6.5 yrs of age) completed questionnaires that collected information about their sleep quality characteristics (Pittsburgh Test, PSQI), chronotype (Horne and Ostberg Questionnaire) and cognitive functions (Digit Symbol Substitution Test, DSST). Each student performed the DSST at two different times of day: morning (09:00-10:00h, DSSTm) and evening (16:30-17:30h, DSSTe). Gender differences were not found in the statistical analysis. Of the total participants that answered the Horne and Ostberg Questionnaire, 47.5% corresponded to intermediate chronotypes, 45.9% to morning-type and 6.6% to evening-type. Although primarily determined genetically, chronotype changes with advancing age towards a more morning-type orientation, as we can observe in our results. The mean PSQI score was 6.15 ± 1.6, and the rates of poor sleep quality (PSQI score > 5) were 46.8%, specifically the sleep latency (1.86 ± 0.15) and sleep disturbances (1.41 ± 0.09) scores were increased in these participants. No significant difference ($t = 1.17$, $p = 0.26$) was found between mean DSSTm and DSSTe scores (45.32 ± 2.08 and 41.46 ± 1.71, respectively). The students reached regular mean DSST scores for old healthy adults (38.8–66.8). Any significant association was found between sleep, chronotype and cognitive function among senior university students.

Chronotype, sleep quality, cognitive function

P2-24

TEMPERATURE AND LIGHT SENSITIVITY OF STEROIDS AND NEUROSTEROIDS-SYNTHESIZING PATHWAYS DURING THE ONTOGENESIS OF THE SENEGALESE SOLE (SOLEA SENEGALENSIS)

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Flatfish metamorphosis represents a complex process that occurs during early development and is accompanied by huge anatomical, physiological and behavioural transformations. This metamorphosis implies a migration of animals from pelagic to benthonic habitats that determines marked changes in temperature and light intensity and spectrum to which they are exposed at critical developmental stages. Although light and temperature effects on flatfish metamorphosis have been reported, their action on early steroidogenesis has not yet been approached. Therefore, the aim of the present study was to elucidate how temperature and light daily cycles and

spectrum affect the expression and rhythms of steroids/neurosteroids-synthesizing enzymes during early development and metamorphosis of the Senegalese sole, *Solea senegalensis*. Fertilized eggs of Senegalese sole were obtained at one day post-fertilization (dpf), distributed on 6 cylindrical tanks of 150 L, and reared under 6 experimental conditions of light and temperature: natural thermocycle (TC) and white light/dark cycle (LDW); TC and red light/dark cycle (LDR); TC and continuous white light (LL); constant temperature (CTE) and LDW; CTE and LDR; and CTE and LL. Larvae were sampled at hatching, pre-metamorphic, metamorphic and post-metamorphic stages and at six different daily points. The specimens were euthanized, quickly frozen in liquid nitrogen and stored at -80°C until used. Subsequently, RNA was extracted and retrotranscribed for real-time quantitative PCR analysis of different steroids/neurosteroids-synthesizing enzymes (*star*, *3 β hsd*, *17 β hsd*, *cyp19a*, *cyp19b*, *cyp17b*) and steroid receptors (*ar α* , *ar β* , *er α* , *er β*). Our results showed that both temperature and light conditions affected the steroidogenic system of sole during the ontogeny and, particularly, during the metamorphosis. Changes in light and/or temperature cycles and light spectrum disturbed the daily expression rhythms of many of the steroid-related genes analyzed, being the effects of light modification much more evident. In turn, *cyp19b* (brain aromatase) was one of the most affected gene. Taken together, our results suggest that the ontogeny of the steroidogenic and neurosteroidogenic systems is highly sensitive to light and temperature regimes. Considering the importance of early steroids and neurosteroids in sex differentiation, neurogenesis, neuronal growth, synaptic formation, brain plasticity and behavior, our results reinforce the importance of the use of natural light and temperature cycles at early rearing stages for a proper development of fish. Funded by MINECO Grants (AGL2013-49027-C3-2-R, AGL2017-82582-C3-1-R) to JAMC.

Ontogeny, steroids, neurosteroids, temperature, light, rhythms

P2-25

ONTOGENETIC EXPRESSION RHYTHMS OF VISUAL OPSINS IN SOLEA SENEGALENSIS LARVAE ARE MODULATED BY METAMORPHOSIS, PHOTOPERIOD AND LIGHT SPECTRUM

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In the fish retina, there are two types of photoreceptors: rods and cones. The rods are highly sensitive photoreceptors for vision in nocturnal conditions. The cones contain opsins and depending on the structure, they can perceive long (red), medium (green) or short (blue and UV) wavelengths of light. Cones of different wavelength sensitivity and the consequent pathways of connectivity with the brain are the base of colour perception. Our fish model, the Senegalese sole (*Solea senegalensis*), experiences a metamorphosis during development that involves physical (migration of the left eye into the right side of the head) and behavioural (from diurnal to nocturnal locomotor and feeding activity) changes. Metamorphosis also determines a migration from pelagic to benthonic environments, which is accompanied by important changes in light characteristics (intensity and spectrum). Based on these facts, we hypothesized that the photoreceptors could also undergo changes in the course of development and metamorphosis. In these animals, we

analysed the developmental and daily expression rhythms of different visual photopigments: rhodopsin (RH1), green-opsins (RH2.3, RH2.4A), UV-opsin (SWS1), blue-opsin (SWS2A) and red-opsin (LWS) at hatching (dph0), before (dph7), during (dph17) and after (dph32) metamorphosis. Furthermore, we tested the effect of light spectrum and photoperiod in animals maintained under light-dark cycles of white light (LDW), blue light (LDB), red light (LDR) and continuous white light (LL). Most of opsins exhibited a pre-metamorphic increase in expression, decreased transcript levels during metamorphosis and a further elevation in mRNA levels at post-metamorphosis (except for green-opsins). Daily rhythms of opsins were more robust under LDB and LDW conditions, in particular, before (dph7) and after metamorphosis (dph32), with acrophases at the day-night transition. In LDR and LL conditions, the expression was markedly reduced for most of the opsins analysed. Rhythmic profiles were evident at dph7, but expression peaks move to the beginning of the day (LDR) or at the beginning of the subjective day (LL). After metamorphosis, some of the opsin rhythms disappeared under LL and acrophases were displaced to the beginning of the subjective night. Our results show that the metamorphic transition from pelagic to benthonic habitats is accompanied by a remodelling in opsin gene expression, which is markedly influenced by light photoperiod and spectrum. Our results also suggest that most of the opsins analysed present endogenous circadian rhythms that are maintained under constant light conditions.

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Ontogeny, opsins, photoperiod, spectrum, rhythms, flatfish

P2-26

CHARACTERIZATION OF THE PANCREATIC ALPHA-CELL DURING AGING

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Introduction: The prevalence of glucose intolerance and diabetes increases with aging. While several alterations have been observed in the pancreatic beta-cell function with aging, there is no information about the alpha-cell population. In the present work, we aim to study the potential alterations in the pancreatic alpha-cell and their effects on glucose homeostasis in old mice.

Materials and Methods: 20-month-old C57BL/6J mice were compared with 3-month-old mice. The pancreas of these animals was extracted to perform immunohistochemical staining and morphological analysis. Plasma hormones were measured with commercial ELISA kits.

Results: We found that old mice exhibited glucose intolerance and insulin resistance along with hyperinsulinemia and hyperglucagonemia in fasting conditions. Acute administration of arginine by intraperitoneal injection led to higher insulin and glucagon plasma levels in old mice. While no glycemic differences were found when animals were challenged with an intraperitoneal glucagon injection, administration of pyruvate resulted in a lower glycemic increase in aged animals. Old mice showed an augmented absolute alpha-cell mass, while no changes were observed in the alpha-cell size.

Conclusions: These preliminary results indicate that pancreatic alpha-cells exhibit several alterations during aging that could affect the regulation of glucose homeostasis.

Alpha cell, glucagon, aging

P2-27

IDENTIFICATION OF THE POLYMORPHISMS IN SLC2A2 GENE AND THEIR ASSOCIATION IN A POPULATION WITH DIABETES MELLITUS TYPE II

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Diabetes mellitus type 2 (DMT2) is the most common type of diabetes (90%) and is due to a progressive loss of β -cell insulin secretion, frequently in the context of insulin resistance. Various genetic and environmental factors may be related to the disease. Recently, efforts have focused on the identification of genes that may be associated with the development of DMT2. The Solute Carrier Family gene (SLC2A2) that codes for GLUT2 is key in the metabolism of glucose playing an important role in the pancreas for the synthesis of insulin. A low expression of GLUT2 in pancreatic islet β cells could be due to polymorphisms throughout the promoter region interrupting the normal process of transcriptional initiation of the gene. Polymorphisms at the level of the promoter region of SLC2A2 have been scarcely studied in their relationship with DMT2. Common polymorphisms have been found in the promoter region of Danish, Finnish, Korean and Venezuelan populations related to DMT2. We compared the incidence of sequence variations in the GLUT2 promoter in 55 normal subjects and 48 patients with T2DM in the Venezuelan population. Sequencing of the promoter region (+284 to -510) revealed more frequently 4 polymorphisms -407 A> G, -149 A> C, -122 C> T and -44A>G. Individuals with variations in the positions -44A> G and -407A> G and corresponding to the GG haplotype, respectively, were the most at risk for developing the pathology (OR = 21.6, CI = 1.2-388, P G and -44A> G affects the activity of the promoter, we have decided to carry out in vitro transfection experiments with a luciferase reporter vector. These results suggest that polymorphisms at positions -44 and -407 may affect gene transcription, possibly associated with reduced GLUT2 gene expression in patients with T2DM. A study of this nature would help to understand the relationship of polymorphisms found in the Spanish population with the severity of the disease.

Polymorphism, GLUT2, Diabetes Mellitus, Human

P2-28

DIETARY AND NUTRITIONAL INTERVENTIONS TO IMPROVE ELDERLY HEALTH IN A PUBLIC ELDERLY RESIDENCE

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Introduction: Concerning malnutrition, the old age is one of the most susceptible life stages due to different causes, including physiological, psychosocial and pathological factors. Altogether lead to a vicious cycle of malnutrition and disease, affecting both non-institutionalized and institutionalized elderly people. For next generations, community stakeholders such as public institutions must be aware of the importance of the consumption of a balance diet for our elders to achieve the goal of healthy ageing.

Aims: The main objective of the study was to analyse current diet and design a new healthy diet for the elderly people living on the public elderly residence “Virgen de Guadalupe” (Olivenza, Badajoz, Spain), trying to improve their nutritional status.

Materials and methods: It was analysed their current summer/spring diet (SSD17) and a new summer/spring diet (SSD18) was design, taking into account that 65% of the individuals suffer from Alzheimer’s Disease, 55% hypertension, 35% diabetes and 25% have heart disease. These diets were analysed with the nutritional software DIAL® (v.3.54., 2016, Alce Ingeniería). Statistical analysis was performed using GraphPad Prism® (version 6.0e, 2014; GraphPad Software, Inc; San Diego, CA).

Results: SSD18 provided significantly less energy than the original diet (SSD17) (2894 versus 2520 Kcal, P<0.0001), from a statistical point of view. Related to macronutrients, a significant decrease was observed in the amount of proteins (141.1 versus 104.3 gr, P<0.0001) and lipids (120.7 versus 95.98gr, P<0.0001). Despite not saw any significant differences in carbohydrates levels provided by both diets, there was a clear difference in their caloric profile (50.6% in SSD18 in front of 43.5% in SSD17). Concerning to the lipid profile, it was observed that SFA (42.8 versus 29.5 gr, P <0.0001) and MUFAs (56.0 versus 42.5, P <0.0001) levels decreased, but PUFAs levels increased (12.3 versus 15.6 gr, P <0.0001). Furthermore, cholesterol levels significantly decreased in the new diet (519.5 versus 325.5 mg, P <0.0001).

Conclusion: The elderly residence dietary pattern has been improved increasing the consumption of fruits, vegetables, nuts, low-fat dairy products, fish, and lean meat (based on regional and seasonal food).

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Healthy ageing, nutrition, diet, elderly residence

P2-29

RELATION BETWEEN ADHERENCE TO MEDITERRANEAN DIET AND ANXIETY IN HEALTHY AND ONCOLOGICAL WOMEN

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Introduction: The World Human Organization (WHO) has highlighted the relevance of Mediterranean diet (MD) in prevention of non-communicable diseases, like cancer, and improving mood. This diet is characterized by a high intake of fresh plant food (fruits, vegetables, cereal), olive oil as the principal source of fat, fish and poultry consumed in low to moderate amounts, so as wine, and a low consume of red meat.

Aim: In previous investigations, our research group has found a positive association between an adherence to MD and an improvement of mood. Owing to that fact, our main objective is to clarify whether MD may have a favourable effect on anxiety in our volunteers.

Methods: In our study participated a control group of healthy adult women (N=35) and a study group of women that have overcome a tumour or are being treated for one at present (N=8). These volunteers were from Oncological Association Tierra de Barros (Badajoz, Spain) and aged 30-70 years old. Participants noted their diet in a dietary record during a week and filled Mediterranean Diet Adherence Questionnaire (MDAQ) and Beck’s Anxiety Inventory (BAI). Scores obtained in both test were correlated through Pearson’s Test, and anxiety levels were compared between groups through an unpaired T test (Graphpad Prism v.5).

Results: We observed that the study group showed higher levels of anxiety than the control group (p<0,05). We also found a positive correlation (p<0,05) between anxiety and the score obtained with MDAQ in both groups.

Conclusion: Women who have gone through a disease like cancer show higher levels of anxiety. Moreover, we reported that a higher adherence to MD may have positive effects on anxiety levels in adult women with or without cancer.

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Mediterranean Diet Adherence, Anxiety, Cancer

P2-30

RELATIONSHIP OF DAILY SUGAR-SWEETENED BEVERAGE CONSUMPTION AND LEPTIN IN MEXICAN YOUNG ADULTS

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Objective: The present study aimed to evaluate the relationship between the high consumption of sweetened beverages (SB) and leptin in young adults Mexican.

Design and methods: In the present cross-sectional study, 92 healthy volunteers, between 18 and 35 years old were recruited (47% female). Weight, height and body composition was evaluated by bioelectrical impedance analysis; SB consumption were measured by a validated food frequency questionnaire. Biological samples were collected in 12-h fasting blood samples to determine leptin, by an ELISA kit under manufacturer’s instructions. The Ethics Committee of the Faculty of Nursing and Nutrition, UASLP (CEIFE-2014-092), has approved this study.

Results: 87% of the population presented high consumption of SB (> 150 kcal/day) and 13% moderate consumption (less than 150 kcal/day). 54% on normal weight, 33% overweight and 13% were obese. Results shown no significant differences on SB consumption on gender were found, neither by BMI. Female showed higher values of leptin, compared to male (20.7 ug/mL vs 11.5 ug/mL respectively $P < 0.05$). Serum levels of leptin were significantly higher (15.3 ug/mL) in population consuming more than 150 kcal/day of SB compared to those with a moderate consumption (10.8 ug/mL). Our results showed a positive correlation between leptin levels and SB consumption ($p < 0.05$) also showed a positive association between SB consumption and waist circumference (0.025).

Conclusion: High consumption of SB was associated with higher concentrations of leptin and waist circumference. Female showed higher leptin and no significant differences were observed in SB consumption by gender, neither by BMI.

Sweetened beverages, leptin, BMI

P2-31

RELATIONSHIP BETWEEN URINARY TOTAL POLYPHENOLS, 8-ISOPROSTANE LEVELS AND DIETARY PATTERN

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Background: Dietary patterns have been widely related to the rates of cardiovascular diseases and life expectancy in different population.

Mediterranean Diet is associated with low incidence of physiologic and metabolic non-communicable diseases such as hypertension, obesity and insulin resistance. Since these types of chronic diseases are the major cause of mortality in developed countries, it is important to know the dietary pattern of populations through easy and objective measurements, able to improve data obtained from different types of questionnaires, such as food frequency questionnaires, dietary recall, and diet diaries.

Objectives: The aim of this work is to establish the relationship between the dietary pattern, the total intake of polyphenols with the diet and the levels of polyphenols in urine, estimated by the Folin-Ciocalteu method, and the lipids peroxidation measurement by the urine 8-isoprostane levels.

Material and methods: In this trial we recruited healthy volunteers from students of the University of Jaén ($n=100$) aged 19-55 years, 89% women and a BMI of 23 ± 0.4 . Potential participants were excluded if they had clinically significant illness and women when they were pregnant or breastfeeding. Each participant completed a food frequency questionnaire (29 items) and a 24h food diary during three days. The intake of polyphenols was estimated using the phenol explored data base. In order to measure the total amount of polyphenols and 8-isoprostane levels, a sample of urine from each participant was collected.

Results: The main source of dietary polyphenols were fruit, vegetables, pulses, nuts and virgin olive oil. There was a significant and positive correlation between the estimate intake of polyphenols with the diet and the total polyphenols excreted in urine ($r=0.2248$; $p < 0.001$), and the intake of different groups of foods (fruit $r=0.8920$ $p < 0.05$; vegetables $r=0.6473$ $p < 0.001$; nuts $r=0.3786$ $p < 0.001$). However, a significant but negative correlation was established between the total intake of polyphenols and the amount of 8-isoprostanes in urine ($r=-0.2357$ $p < 0.001$).

Conclusion: The measure of total polyphenols by Folin-Ciocalteu method and the levels of 8-isoprostanes in urine have the potential to be used to assess adherence to dietary patterns and the intake of antioxidants nutrients.

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Diet, urine, polyphenols, 8isoprostanes

P2-32

HIGH FAT DIETS AND CHANGES IN THE HYPOTHALAMIC PROFILE OF ANGIOTENSINS. EFFECTS ON BODY WEIGHT AND ADIPOSITY

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Background: Obesity is one of the most important nutritional problems in developed countries, and it has been widely associated with an increase in the prevalence of hypertension and cardiovascular diseases which are the primary cause of worldwide mortality. The local Renin-Angiotensin-System (RAS) in the brain has been related not only to water intake and blood pressure control, but also to the regulation of body weight and energy homeostasis. Moreover, the main peptide in this system (AngII), affects to the sympathetic activity and heat production, as well as to the metabolism of oxytocin, a peptide implicated in the control of feeding behaviour. On the other hand, high fat diet had demonstrated to affect hypothalamic AngII levels, and this effect seems to be related to energy imbalance. However, not all fat sources show the same effects on health.

Objective: The main goal of this work was to analyse the possible effects of two high fat diets, with different fat source, on hypothalamic

angiotensin profile and oxytocin levels, and their relationships with body weight gain and adiposity.

Material and Methods: Male Swiss Webster mice were divided in three groups (n=9). One group was fed with a standard chow diet (Control group), and the other two with a high fat diet, supplemented with different sources at 20%: butter (Butter group) or Virgin Olive Oil (AOVE group). Animals were fed during three months in ad libitum conditions and, at the end of the experimental period, were sacrificed under anaesthesia, and samples of hypothalamus were obtained. Angiotensin profile was determined by nano-HPLC MS/MS and oxytocin using an ELISA commercial kit.

Results: Only Butter group showed high body weight and high amount of visceral adipose tissue. When samples of hypothalamus were analysed, AngII and AngIV were significantly lower in both AOVE (p<0.01) and Butter groups (p<0.01) than in controls. However, the amount of AngIII was also lower than controls in AOVE animals (p<0.001), but similar in the Butter group.

Conclusion: Taken together, these results support the role of hypothalamic RAS in the development of obesity induced by diet, and the specific effects of different fat sources related to a different metabolism and profile of brain angiotensin peptides.

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Obesity, RAS, oxytocin, olive

P2-33

LIFE-LONG FEEDING DIETS BASED ON DIFFERENT UNSATURATED FATS, SUPPLEMENTED OR NOT WITH COENZYME Q, DIFFERENTIALLY MODULATE LIFESPAN IN MALE WISTAR RATS

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The role of dietary fat and antioxidants in aging has been extensively investigated for the last decades. Research has shown that certain unsaturated dietary fats, such as virgin olive or fish oils, and some antioxidants like coenzyme Q (CoQ), positively affected different aspects of the physiological process of aging. Therefore, it might be possible that such positive aspects on aging could also modulate an intimately related parameter as lifespan. To evaluate this hypothesis, 150 male Wistar rats (*Rattus norvegicus*) weighing 80–90g were randomly assigned into six experimental diets from weaning until natural death. Experimental treatments were semi-synthetic and isoenergetic diets prepared according to the AIN93 criteria. These diets differed both in the fat source (virgin olive, sunflower or fish oils) and in the presence or not of CoQ10 (50mg/kg per day). Individual follow-up was performed for the whole life of the animals. Survival time was defined for each individual as the time (in days) elapsed from the date of beginning of study to the date the individual was found dead. Some situations, particularly accidentally deaths, were considered censored data (a total of six animals). Data were analysed using SPSS statistical software package (SPSS for Windows, 24.0 SPSS Inc.

Chicago, IL, USA). Survival probability distributions for each dietary treatment were estimated by the non-parametric Kaplan-Meier estimator and comparisons between diets were performed following Log-rank, Breslow and Tarone-Ware estimation tests, assuming a significance level of 0.05. For non-supplemented groups, Log-rank test revealed that survival probability was lower in rats receiving sunflower oil respect to those fed virgin olive oil at any age, but no differences were found between sunflower and fish oil-fed animals with this test. Differences between fish and sunflower oil fed animals were found statistically significant according to Wilcoxon–Breslow and Tarone-Ware tests. When dietary fats were compared on the basis of supplementation or not with CoQ10, statistically significant differences were found only between sunflower oil fed animals, both for Wilcoxon–Breslow and for Tarone-Ware tests. In conclusion, these results suggest that life-long feeding on virgin olive oil enhanced survival probability at all stages of life when compared with animals fed on sunflower oil. Moreover, fish oil consumption would also improve survivorship compared to sunflower oil but only at particular life periods. Finally, CoQ10 supplementation would improve survivorship, but only for sunflower oil fed animals.

Aging, Olive, Sunflower, Fish

P2-34

EXERCISES IMPROVES HFD-MEDIATED METABOLIC SYNDROME, DISBIOSIS AND ASSOCIATED GUT-LIVER AXIS AND BILIAR ACIDS Deregulation IN AN IN VIVO MODEL OF EARLY OBESITY AND NAFLD

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Introduction: Childhood obesity have reached epidemic levels representing one of the most serious public health concerns associated with metabolic syndrome, nonalcoholic fatty liver disease (NAFLD) and gut microbiota alterations. There is few clinical experience for pediatric NAFLD patients, and since no perfect NAFLD in vivo model has been developed, its pathogenesis remain unclear and the therapeutic options are very scarce with respect to safety, effectiveness, and patient compliance. Physical exercise is known to improve obesity and NAFLD progression, modulating the gut microbial balance.

Aims: To evaluate the benefits of exercise on gut microbiota and metabolic status modulation in an in vivo model of early obesity and NAFLD.

Methodology: 21 days old male Wistar rats fed with control (C) or high fat diet (HFD) were subjected to an interval aerobic training protocol. Fecal microbiota was sequenced by Illumina MiSeq system and parameters related to metabolic syndrome biliar acids and gut-liver axis alteration were measured.

Results and conclusions: Exercise decreased HFD-induced body weight gain, metabolic syndrome, liver dysfunction, and intrahepatic lipid accumulation as a result of its lipogenic metabolism modulatory capacity. Exercise training also reduced the subsequent lipotoxicity and improved the inflammatory response, downregulating the NF-κB transcriptional activity induced by HFD. Some of this effects seems to be mediated by its capacity to preserve intestinal barrier functionality, which in turn prevents gut-liver axis deregulation and improves the bile acids enterohepatic circulation and homeostasis. Besides, exercise effectively counteracted HFD-induced dysbiosis, increasing the Firmicutes/

Bacteroidetes ratio vs sedentary rats. Comparison of the microbiome at phylum level indicated that the bacterial communities grouped separated according to different factors: diet, age, and exercise. Similarly, these factors were found to influence the composition at genus level. We provide scientific evidences highlighting the benefits of physical exercise protocols to modulate the intestinal microbiota in the management of childhood obesity and NAFLD development, via its anti-inflammatory, lipids and biliar acids metabolism modulatory and prebiotic capacities. Supported by LE063U16 (JCyL and FEDER), BFU2017-87960-R, GRS1428/A/16 and CIBERehd funded by ISC III.

Childhood obesity, exercise, NAFLD, intestinal microbiota

P2-35

UPDATING THE SPANISH DATA BASE FOOD COMPOSITION (BEDCA): IMPLEMENTATION OF THE VERSION 2.3

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Introduction: Food composition databases (FCDB) are important tools for food and nutrition professionals (1). Food composition data can be obtained from various sources. Different proposals exist to evaluate the quality of these data (2).

Methods: 1. Food description and coding systems: a) FoodEx2 (FoodEx2 Browser -user's guide, EFSA 2017). b) LanguaL (2008 version). 2. Scientific literature. 3. Excel software (data and metadata). 4. BEDCA (version 2.2). 5. SPSS package (version 22.0).

Results: Actually, BEDCA provides information about a total of 950 foods, with 48 components (macro y micronutrients, included a list of individual fatty acids, trans fatty acids, mono and disaccharides, non-provitamin carotenoids, the classification of foods, based on their content of natural sugars and / or added. The profile of vitamins and minerals was also completed, 'controversial' data were reviewed, the specification of foods with and without gluten of different commercial brands. We have recoded and reclassified with the latest version of FoodEx2, after the changes imposed by EFSA recently (FoodEx2 Browser -user's guide, 2017) all the foods included in BEDCA. This has meant a comprehensive, expensive and long-lasting work. We have developed a guide-tutorial of FoodEx 2 employment in Spanish, for use by postgraduate students, in specific courses, on tools and methods for nutritional studies of the University of Granada. We have finalized the process of inclusion of BEDCA in EVALFINUT (<http://www.finut.org/evalfinut/>), a computer application developed by the Iberoamerican Nutrition Foundation (FINUT), for evaluation or design of diets. The composition data and their coding in LanguaL and FoodEx2 of new meat foods, provided directly by industries, previously analyzed in the Food Analysis Unit of the UGR, have been included. We have expanded the list of Spanish foods from the latest food surveys, not included in BEDCA. We have compiled and included in BEDCA analytical data of rich foods FODMAP, in order to establish information alerts, for patients with inflammatory bowel disease. Synergies have been established with relevant companies in the Spanish market, allowing us to analyze and include

BEDCA's own data on numerous white label foods, which are very consumed in Spain;

Conclusions BEDCA is currently the only BDCA developed in Spain with documented and compiled data, according to EuroFIR standards and included in FoodExplorer. BEDCA allows users and professionals in Nutrition and Public Health the online use of quality food composition data.

EuroFIR, BEDCA, EVALFINUT

P2-36

NUTRITIONAL EVALUATION AND RISK OF EXPOSURE TO CONTAMINANTS THROUGH THE DIET, IN STUDENTS OF THE UNIVERSITY OF GRANADA

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Introduction: The relationship between chronic noncommunicable diseases (obesity, cardiovascular disease, metabolic syndrome, type II diabetes) and the consumption of some foods, or more specifically of some of the components of those foods (fat, simple sugars, contaminants...) is well known. (1,2). The university population is attributed a series of inappropriate eating habits and far from what is known as the Mediterranean Diet (DM), which are accompanied by nutritional deficits (vitamin, minerals, fiber) and an unbalanced caloric distribution (3).

Objetives: Evaluate the nutritional status of the population studied, determine the intake of contaminants through diet and relate them to adherence to the Mediterranean diet.

Method: The population studied in this study has included 40 students from the University of Granada (UGR). The students completed different questionnaires for the dietary evaluation: a) Food Consumption Frequency Questionnaire (CFCA), b) PREDIMED questionnaire, to study the adherence to DM, c) Repetitive reminder of 24 hours (7 days, 1 festive). The nutritional evaluation was carried out through the Food and Health program, version 2.0 and the evaluation of the intake of contaminants through Ribefood (2007). The anthropometric measures were: height (m) and weight (kg) and body mass index (BMI).

Results: The results of the subjects surveyed (19.65 ± 1.79 years, 57.5% women versus 42.5% men.) Showed an insufficient intake of calories, low carbohydrate consumption, correct saturated fatty acids (AGS), excessive of proteins, fats and polyunsaturated fatty acids (AGP), and deficient of monounsaturated fatty acids (AGM). The insufficient intake of some vitamins (A, B5, biotin and B9) and minerals (potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) (especially in the female population), zinc (Zn)), iodine (I), copper (Cu) and chlorine (Cl) and fiber. Regarding the intake of contaminants, only 12.5% of the population exceeds the maximum allowable intake of some, with the largest contributors of total Hg and the group of dioxins, swordfish and anchovies, respectively. According to the questionnaire of Prevention with DIET MEDiterránea (PREDIMED) (14 items), 30% of the students presented a high adherence to the DMe, 65% an average adherence and 5% a low adherence. Of the 5 students who have exceeded the maximum admissible intake established for some pollutants, 4 had a high adherence to the DMe.

Conclusions: It is necessary a nutritional intervention in the university population, to improve the intake profile of certain nutrients, increase adherence to the Mediterranean diet and avoid excessive intake of contaminants through the diet.

Mediterranean Diet, Contaminants, Students, University

P2-37

EFFECT OF GLUCOSE CONCENTRATION ON THE ACTIVITY OF UBIQUITIN E3 LIGASE OF MDM2

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The degradation of p53 occurs via ubiquitin-26S proteasome, after Mdm2 (murine double minute 2) marks p53 with ubiquitins. The concentration and phosphorylation of Mdm2 and p53, greatly affect the ubiquitination rate of p53, by avoiding the recognition and physical interaction between p53 and Mdm2. Phosphorylation of Mdm2 in Ser395 by ATM decreases its ability to direct the degradation of p53. Therefore, we aim to study the effects of high glucose concentrations on the RING domain of Mdm2 and its ubiquitin E3 ligase activity. We cultured RINm5F cells in RPMI-1640 medium supplemented with 10% fetal bovine serum (v/v) at two concentrations of glucose 5 and 30 mM for up to 72 h, in a humid atmosphere at 37 °C and 5% CO₂. At the end of the treatment the cells were lysed to separate the nuclear and cytosolic fraction. After quantifying the proteins, they were visualized on SDS-PAGE and transferred to a PVDF membrane. Primary antibodies against Mdm2, Mdm2 p-Ser395, ATM, Arf, c-Abl, and ubiquitin were used and revealed using an amplified fluorescence kit from GE. The results show that the stress caused by high concentrations of glucose stimulates the phosphorylation of Mdm2 in Ser 395 by ATM activation. The phosphorylation of Ser395 alters the RING domain of Mdm2 and decreases its activity of ubiquitin E3 ligase, ubiquitination and degradation of p53. Therefore, stress due to increased glucose decreases the activity of ubiquitin E3 ligase of Mdm2 by inducing the phosphorylation of this residue, which leads to the stabilization and increase of p53 and the subsequent activation of apoptotic events. The Ser 395 residue of the RING domain is important for the efficient function of ubiquitin ligase of Mdm2 in β cells under conditions of hyperglycemia.

Mdm2, Ubiquitin E3 ligase, Apoptosis, Phosphorylation

P2-38

METABOLIC SYNDROME IN THE BINGE DRINKING

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Introduction: It is reported that alcohol consumption and more recently, binge drinking (BD), increases the risk of developing the metabolic syndrome (MS); however there are few studies on this relationship and even less in adolescent population. On the other hand, recent study supports the role of AMP-activated protein kinase (AMPK), a key energy sensor which is essential to have a good metabolic homeostasis, in MS pathology.

Objectives: Study the effect of BD on some risk factors of MS and analyze the implication of AMPK protein in this metabolic disorder.

Methods: It was used adolescent rats divided in two groups: control and

BD groups. Alcohol solution (20% v/v) was administered intraperitoneally (3 g/Kg/day during 3 days/week in 3 weeks) to BD group. Control group received an intraperitoneal injection of an equal volume of isotonic saline solution during the same time. Weight gain, abdominal and toracic circumference, metabolic profile, as well as hepatic AMPK activation were measured.

Results: BD group had larger abdominal circumference and higher fasting serum glucose, triglycerides and cholesterol concentrations although they showed a weight gain similar to control rats. Also, BD group presented a higher expression of total AMPK and lower expression p-AMPK that control ones.

Conclusion: BD decreases the activation of AMPK, which alters the metabolism of lipids and glucose and leads to abdominal obesity. These results suggest that BD promote some risk factors for MS.

Binge drinking, adolescent, metabolic syndrome, AMPK

P2-39

RELATIONSHIP BETWEEN DIET AND SOCIAL COGNITION IN UNIVERSITY STUDENTS

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Introduction: Nowadays, many diseases as diabetes, obesity, hypertension or hypercholesterolemia can be prevent, treat or cure through healthy dietetic patterns. In the last years, a close relationship between nutrition and cognition has been reported. It is also known that diet may influence mood and different mental processes having a positive influence on quality of life.

Aims: The main objective of this study was to evaluate the relationship between macronutrients provided by diet and the processing of social and affective information.

Materials and methods: Participants were 117 undergraduates from the University of Extremadura (Badajoz, Spain) aged 21.3 ± 2.9 years old, with a mean Body Mass Index of 22.8 ± 3.9 (kg/m²). Students answered the following psychological questionnaires: WHO-5 Well being Index, Beck Anxiety Inventory, Beck Depression Inventory, Ruminative Responses Scale (RRS), Interpersonal Reactivity Index (IRI), LEIDS-R, and Empathy Quotient. In addition, they filled out a dietary record for a week and noted all ingested food and beverages. These diets were analysed with software DIAL v.1.18® (Alce Ingeniería). First of all, nutrients were grouped through a Principal Components Analysis (into lipids, carbohydrates and proteins). Additionally, we assayed if these Principal Components were associated with psychological test score using multiple linear regression analysis. Statistics were performed with SPSS v.24® and G*power v.3.1.9.3®.

Results: Perspective-taking Scale (IRI) was affected by lipids (p <0.05; R=0.27; f²=0.08; Power= 0.66), specifically by cholesterol (p<0.05; R=0.29; f²=0.09; power=0.65). On the other hand, carbohydrates influenced the Depression Factor (RRS) (p<0.05; R=0.32; f²=0.11; power=0.82), seeming that complex carbohydrates may be the main cause for this (p=0.07; R=0.30; f²=0.09; power=0.75). Moreover, the Reproach Factor (RRS) was affected by dietary carbohydrates and proteins (p<0.05; R=0.32; f²=0.11; power=0.82), specifically by fiber (p<0.05; R=0.28; f²=0.09; power=0.69) and by aspartate (p<0.05; R=0.51; f²=0.15; power=0.62).

Conclusions: Diet may have an influence on mood and social cognition. In particular, diet rich in fiber, aspartate and poor in cholesterol, may provide benefits improving the processing of social and affective information.

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Social cognition, mood, lipids, carbohydrates, proteins

P2-40

CAENORHABDITIS ELEGANS AS A MODEL TO INVESTIGATE ANTI-DIABETIC EFFECTS OF NOVEL DESIGNED AND SYNTHESIZED COBALT COORDINATION COMPOUNDS

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Diabetes mellitus is a disease that manifests when the pancreas does not produce enough insulin (type I) or when the body cannot effectively use the insulin it produces (type II). Some metal ions have shown insulin-like effects by supporting the signal transduction of insulin and reducing the production of cytokines, triggering a cascade of events leading to beta-cell death during the pancreatic inflammatory process in the course of disease. Coordination compounds have demonstrated to inhibit enzymes that play a major role in modulating insulin sensitivity and might be an alternative to traditional insulin therapy. The nematode *Caenorhabditis elegans* has proven to be an important animal model to study the molecular mechanisms of drug effects and disease pathogenesis. Many key findings with relevance for mammals were discovered in this well-characterized model organism, which is possible because of the strong conservation of biological principles between *C. elegans* and mammals and because 60–80% of human gene homologues have been identified in *C. elegans*. In this study, we tested two novel mononuclear coordination compounds [Co(bumetanide)₂(H₂O)₂](H₂O)₂, or compound 1, and [Co(indomethacin)₂(EtOH)₂], or compound 2, both synthesized using conventional routes to achieve their potential for reducing glucose levels. Results from the present study shows that exposure of nematodes to increasing concentrations of compound 1 resulted in a lack of acute toxicity, expressed by the lethality test, up to a dosage of 200 μM. For compound 2, a statistically significant lethality level was only showed for 200 μM, the highest tested dosage. According to these results, authors decided to test anti-diabetic potential of assayed compounds by using the highest and non-toxic dosages for any of the two compounds, i.e. 100 μM dosage. Results showed that compound 1 presented statistically significant lower glucose levels that control group (mean reduction of 31%, $P < 0.05$). In the same way, compound 2 reported lower glucose than nematodes from the control group (24% less glucose, $P < 0.05$). According to these results, we can conclude that both, compounds 1 and 2, might be endowed with anti-diabetic properties, since one of the main features of diabetes is an excess of glucose in the organism, irrespective of the cause of this excess. The present research demonstrate the potential of the use of a high-throughput screening model like *C. elegans* to test cobalt-based newly designed compounds for the putative treatment of altered glucose metabolism-dependent diseases.

Glucose, chemistry, diabetes

P2-41

EFFECTIVITY OF PHYSIOTHERAPY TREATMENT IN PIANIST WITH DE QUERVAIN'S DISEASE. BIBLIOGRAFIC REVIEW

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Introduction: the upper extremity injuries' are a big impact in the society, having a high frequency and causing economic consequences, and disability. Pianist are a group of people with tendency to have musculo-eskeletal loads due to playing many hours and the psychological stress cause by a high level of exigency. All excessive use of upper extremity could be produce a disease. Principal diagnosis in this type of syndromes, are De Quervain's disease and affected two structures: the large abductor and the short extensor of the thumb. For this point, I consider that it is completely necessary to do a bibliographic review with the aim to know if exist and efficacy physiotherapy treatment for this pathology.

Materials and methods: a bibliographic search, about the results of physical treatments in comparison with others treatments and the use of physiotherapy in combination with other disciplines or inside of multidisciplinary treatment, was made in principal databases like Medline, Scopus, Web of Science, PEDro and The Cochrane Library.

Results: After the search, a critical reading of 13 articles was realized about the physiotherapy approach like deep transversal massage, ultrasound, kinesio-tape or excentric exercise and the mobilizations of the thumb in this type of patients. These results are very effective and have some positive results, and these results are better when combined more than one no-surgical treatments (corticosteroids injects, orthopedic treatment or nonsteroidal anti-inflammatory) before the surgical treatment (only use when the others treatment did not perform).

Conclusions: the physical or physiotherapy treatment of De Quervain's disease, is considered an efficacy option despite of actually evidence. Although, the best option at this moment to treat this injury is the multidisciplinary treatment due to the combination of different treatments.

Quervain's disease, physiotherapy, pianist

P2-42

CHANGES IN THE HUMAN LACHRYMAL PHYSIOLOGY DUE TO THE USE OF CONTACT LENSES WITH BLUE-LIGHT BLOCKING FILTER

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In the era of information technology, more and more eye problems are detected due to the abuse of the electronic devices with LED-backlit screens. One of the most severe problems caused by these screens is the symptomatology of dry eye, which can appear after one hour using any of these devices¹. This symptomatology, largely, owes to itself to the decrease of the blink rate and the increase of incomplete blinking², that in turn favors the break of the tear film. Currently, in order to tackle these problems, there are filters that block a certain percentage of blue light and therefore improve this symptomatology, and there is an inverse relationship between the blue light rate that the filters can absorb with the reduction of the rate blinking³. This type of filter had been added to ophthalmic

lenses or intraocular lenses, but recently they have been added to contact lenses (CL). On the other hand, the very fact of placing a CL in the ocular surface, divides the tear into two, leaving a thin layer of the tear film to the outside that evaporates easily, worsening the symptoms of the dry eye during the use of visual display terminals (VDTs)⁴. In order to determine whether a blue-light blocking filter incorporated in a CL improves dry eye symptomatology that appears in CL users exposed to VDTs, we compared the: i) tear breakup time and, ii) tear volume, in people who used CL with a filter which blocks 5 % of the blue light with those CL users whose CL did not have any blue light filter. Both parameters were decreased in CL users, and no significant differences were observed between both types of parameters in CL users. Nevertheless, CL users with blue-light blocking filter showed values closer to the normal than those using CL without any filter. In addition, factors such as the filters light absorption rate must be taken into account in future studies.

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Tear, contact lenses, blue-light blocking filter

P2-43

INFLUENCE OF THE USE OF CONTACT LENSES WITH BLUE-LIGHT BLOCKING FILTERS ON CONTRAST SENSITIVITY IN HUMANS

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Nowadays, digital devices are widely present in people everyday lives, having led to some changes in their habits. Consequently, as the number of digital users has increased, there are new risks for our eyes and new challenges for vision care professionals. These digital devices emit blue light, an extremely energetic radiation able to reach the retina almost completely¹. Although the physiological lens (cornea and crystalline), the macular pigment and the foveal blue scotoma prevent us from the risks of blue light², its lasting use and the proximity to our eyes in which we usually use them have brought an increased need for an additional artificial protection based on the use of filters³. Blue light seems to have short-term risks that might give rise to asthenopic symptoms, as well as long-term risks that could cause incurable eye diseases in the elderly, such as Age-Related Macular Degeneration (AMD)⁴. However, due to the fact that light reaching cerebral cortex can be modified by filters, visual performance can be affected by the use of specific light blocking filters. The main goal of this study is to compare visual performance of digital users while wearing blue light filter contact lenses with those who wear non blue-blocking contact lenses after 3 hours exposure to a digital device. For that purpose, contrast sensitivity has been examined at 4 frequency levels (3, 6, 12, 18 cycles per degree) and in two lighting conditions (photopic and mesopic). In conclusion, the blue-light blocking filter used in this investigation seems not to change users' visual performance in terms of contrast sensitivity, possibly due to a low blue light absorption capacity of the filter.

⁽¹⁾ Behar-Cohen et al. (2011) Prog Retin Eye Res 30:239-257. ⁽²⁾ Boulton et al. (2013) International Review of Ophthalmic Optics. ⁽³⁾ Tanito et al. (2018) Adv Exp Med Biol 1074:53-60. ⁽⁴⁾ Edwards and Gibson (2010) Clin Exp Optom 93:390-399.

Blue light, selective filter, contrast sensitivity

P2-44

COMPARATIVE STUDY OF OCULAR LUBRICANTS REGARDING PATIENTS SUFFERING FROM DRY EYE

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Purpose: To evaluate symptomatological changes about dryness in the eye after instilling ocular lubricants. Another purpose is to decide which ocular lubricants will achieve greater benefits.

Methodology: This study involved 27 patients who are students at University of Seville. They participated voluntarily. They suffer from dryness in the eye with symptoms such as a gritty sensation in the eye, itchy, red or watery eyes. They were tested for Schirmer, BUT and OSDI test. After that, they applied Aquoral Forte in the right eye and Aquoral Lipo/Lumixa in the left one. Then, we repeated the after-treatment proofs.

Result: 25 patients completed the study. In the prior treatment, variables were not statistically significant since the value $p > 0.05$. After treatment, Schirmer test resulted in an average value of 26mm in OD and 24.24 mm in OI. BUT test resulted in 14.28 seconds in OD and 12.88 seconds in OI. Both results were higher in comparison with the results got in the prior treatment. However, the average value in OSDI test decreased by up to 10.81. All variables were statistically significant since the value $p < 0.01$. After treatment, the samples were not statistically significant so they were homogeneous.

Conclusion: Both ocular lubricants, Aquoral Forte and Aquoral Lipo/Lumixa, are very effective methods to reduce the symptoms of dryness in the eye since they lubricate, moisten and stabilize the tear film.

Tear film, dry eye, ocular lubricants

P2-45

ONCOLYTIC VIROTHERAPY: VESICULAR STOMATITIS VIRUS AS AN EFFECTIVE TREATMENT AGAINST CANCER

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Oncolytic virotherapy is an anti-cancer approach that has made possible to engineer designer therapeutics, like viruses, specifically targeted to have a greater efficacy and fewer side effects than wild-type viruses. Several oncolytic viruses (OVs) are in clinical trials, others have already been approved, whereas others are being modified to improve them even more. VSV is a promising OV since it is well studied, has an easy-to-manipulate genome, uses a ubiquitous receptor, and lacks pre-existing immunity in humans. VSV is currently being tested in the USA in several phase I trials against different malignancies. Numerous approaches have been published addressing modifications to VSV, generally improving the virus safety, oncoselectivity and oncotoxicity, preventing premature clearance of the VSV from the immune system and resistance of cancers to VSV or stimulating tumor-specific immunity. Multiple approaches are based on combining VSV with chemicals, genetic strategies or other OVs. Otherwise, further modifications need to be done to solve problems that still remain, for example, cancer recurrence, patient toxicity, delivery methods, and safety.

P2-46

THE LEVEL OF MONOENES IN THE DIET MODULATES THE HEPATIC EXPRESSION OF SCD-1b IN THE EUROPEAN SEABASS (*Dicentrarchus labrax*)C. Pendón^{(1)*}, A. González⁽¹⁾, I. A. González^(1,4), J. M. Igartuburu⁽²⁾, G. Mourente⁽³⁾.

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Introduction: One of the great challenges facing today's aquaculture is the search for alternatives to the traditional sources used to prepare the feed used in fish feed. So far, diets are based on two main components obtained from extractive fishing: flours and fish oils. The overexploitation of fisheries worldwide shows that this source is not sustainable. However, the use of alternative sources involves making a rational design of new feeds, formulated with components of different origin. The use of vegetable oils, with a higher content of monoenes and essential fatty acids as an alternative to fish oils, raises serious doubts about the ability of marine fish to satisfy their nutritional needs of polyunsaturated fatty acids (PUFAs) Arachidonic (AA), Eicosapentenoic (EPA) and Docosahexaenoic (DHA) acids, being able to be compromised its ability to synthesize PUFAs.

Objective: In this work we have analyzed the influence of the monoenes content of diets formulated with vegetable oils, on the expression of the hepatic enzymes Stearoyl CoA Desaturase (SCD 1b), the fatty acid desaturase with the highest level of expression in different tissues, and the $\Delta 6$ Fatty Acyl Desaturase ($\Delta 6$ FAD), a enzyme involved in the synthesis of polyunsaturated fatty acids, with a low level of expression.

Methodology: Total RNA was obtained from the liver of 5 individuals per diet tested and retrotranscribed to cDNA. The analysis of the expression of *scd 1b* and *$\Delta 6$ fad* was performed by RT-qPCR in triplicate (n=5), using a absolute o relative (Δ CT) quantitation method. The expression results obtained were statistically contrasted. **Results:** Our results show that the level of expression of *scd 1b* is higher than that of *$\Delta 6$ fad* in all analyzed tissues. The liver showed the highest expression levels in *scd 1b* in contrast to the intestine that shows the lowest levels. Adipose tissue shows the highest *scd 1b*/ *$\Delta 6$ fad* expression rate suggesting a preponderant role of *scd 1b* in fatty acid desaturation. In contrast, in the intestine the ratio shows the lowest, taking the *$\Delta 6$ fad* a more relevant role. We found an inverse correlation between the monoenes content of the diet and the hepatic expression of *scd 1b* and a soft direct correlation of the n-6 (C18:2 + C20:3) content of the diet with the expression of *$\Delta 6$ fad* in European seabass.

Conclusion: The hepatic expression of *scd 1b* show a strong direct correlation with the monoens content of diet and liver. The hepatic expression of *$\Delta 6$ fad* shows a soft direct correlation with n-6 (C18:2 + C20:3) content in the diet but not with that of the liver. Variations in the relative transcription rate (RTR) of both genes suggest that there is a mechanism to control the expression of both enzymes, which allows the control of the level of unsaturated fatty acids in the liver.

SCD1b, $\Delta 6$ FAD, Monoenes, PUFAs, European seabass

P2-47

THE $\Delta 6$ FAD ACTIVITY IS CONDITIONED BY ALTERNATIVE SPLICING IN THE EUROPEAN SEABASS *Dicentrarchus labrax*.Pendón, C.^{(1)*}, González, I.A.^(1,3), A. Pérez⁽¹⁾, Mourente, G.⁽²⁾.

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Introduction: The $\Delta 6$ Fatty Acyl Desaturase ($\Delta 6$ FAD) is a enzyme that introduces double bonds in fatty acids. It is a key enzyme in the pathway of synthesis of polyunsaturated fatty acids (PUFAs) arachidonic (AA), Eicosapentenoic (EPA) and Docosahexenoic (DHA). The marine fish, even having at least one *$\Delta 6$ fad* gene, are unable to biosynthesize AA and EPA from esencial fatty acids (LA and LNA), which makes it impossible to use vegetable oils in diets as a source of fatty acids. **Objective:** In order to study what prevents marine fish to metabolically use Linoleic (LA) and Linolenic (LNA) acids, we have investigated the alternative splicing of the *$\Delta 6$ fad* gene and the influence of the temperature on the $\Delta 6$ FAD mRNA splicing in European seabass (*Dicentrarchus labrax*), a species with high economic value.

Methodology: cDNA obtained from brain mRNA, was amplified with specific primers for $\Delta 6$ FAD. The PCR products were cloned and 18 positives clones were sequenced. The functional analysis of the ORFs were perform in a yeast heterologous system to test $\Delta 5$ or $\Delta 6$ activity. Pairs of specific primers were designed to quantify, by RT-qPCR, the levels of the set of splice variants SV1, 2, 3 and 5, on the one hand, and the splice variant SV4, on the other, in differents adult tissues and in the DLEC embryonic cell line. Temperature effect on alternative splicing was analyzed in DLEC cells.

Results: Five variants of splicing (SV1-5) of the mRNA of the *$\Delta 6$ fad* gene were detected. Taking the larger splice variant SV1 as reference, the splicing occurs at the 5'-UTR in 3 splice variants (SV2, 3 and 5) and in the coding region in another (SV4). Four splice variants (SV1, 2, 3 and 5) encode a protein of 445 aa, with $\Delta 6$ FAD activity. The splice variant SV4 encodes a truncated protein (443 aa) without $\Delta 6$ FAD activity. None showed $\Delta 5$ FAD activity. The RT-qPCR analysis of the expression shows that: a) SV4 splice variant is the most abundant; b) the level of the set of variants SV1, 2, 3, 5 is always higher than that of SV4; c) The temperature promotes an increase of the SV4 greater than that of the set of variants SV1, 2, 3, 5 in DLEC cells.

Conclusion: The mRNA of *$\Delta 6$ fad* gene shows alternative splicing in seabass. Five splicing variants have been detected, being the variant 4 less represented than the set of variants SV1, 2, 3 in all tissues and in DLEC cells. The alternative splicing of the $\Delta 6$ FAD mRNA is influenced by the temperature. The temperature promotes an increase in the SV4 variant greater than the SV1, 2, 3, 5 set. The variation in the level of SV4 associated with temperature suggests that the SV4 levels are not the product of a random splicing effect and, therefore, a possible function of the ORF protein product of the SV4 variant in the regulation of the activity $\Delta 6$ FAD.

$\Delta 6$ FAD, Desaturase, Alternative splicing, Splice variant, RT-Qpcr, seabass.

Poster session 3: Neurodegeneration & Neurogenesis, Clinical & Traslational, Gastrointestinal, Renal & Epithelial, Reproductive, Blood

P3-01

IGF-II AS A NEUROPROTECTIVE AND NEUROPLASTIC FACTOR IN AN OXIDATIVE DAMAGE MODEL INDUCED BY GLUCOCORTICOIDS

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IGF-II is a pleiotropic hormone widely distributed in the CNS, which triggers its functions by binding to IGF-IR, InsulinR and IGFII / M6P (IGF-IIR) receptors. Recently, it has been proposed that the effects of IGF-II, interacting with IGF-IIR, are relevant not only for metabolism, growth and development, but also for neurotransmitter release, memory consolidation and neuroprotection under neurodegenerative processes. The results of our research group prove that IGF-II exerts metabolic, antioxidant and neuroprotective effects in aging. On the other hand, it has shown to have neuroprotective actions in stress-related disorders mediated by glucocorticoids, and even in neurodegenerative pathologies such as Alzheimer's disease or neuropsychiatric disorders. In relation to glucocorticoids, it has been revealed that the exposure of neural cells to high levels or prolonged incubation periods, produce synaptic alteration, neurodegeneration and neuronal death. Mechanisms of glucocorticoid-damage are mediated by oxidative stress induced by an increase in ROS, mitochondrial damage, decrease in antioxidant defenses, lipid and protein membrane damage, etc. AIM: To study the antioxidant and neuroprotective effect of IGF-II in a model of oxidative damage induced by glucocorticoids in aging.

Methods: Primary adult rat neuronal cultures incubated with transient high levels of corticosterone (CORT) in the presence of low concentrations of IGF-II were used. Oxidative damage was evaluated by measuring lipid hydroperoxides and cellular antioxidant status; neuronal function through mitochondrial cellular distribution, and quantification of synaptophysin and PSD95; synaptic functional evaluation with the endo / exocytosis of FM1-43 dye; and neurodegeneration with fluorochrome staining experiments.

Results: Incubation of cells with CORT triggers oxidative damage, consuming antioxidant status. This oxidative stress produces damage and mitochondrial redistribution inducing synaptic changes, as shown the decrease in synaptophysin and PSD95 levels together with a decrease in the uptake and release of FM1-43, which may result in neurodegeneration. Incubation with IGF-II reverses these deleterious effects.

Conclusions: Treatment of cells with IGF-II recovers the damage produced by CORT, restoring synaptic function and decreasing neurodegeneration. These outcomes can be attributed to an antioxidant effect mediated by the interaction of IGF-II with its specific IGF-IIR, which in turn mediates recovery of the redox balance via inhibition of ROS production, improvement of mitochondrial membrane potential / distribution and / or regulation of synaptic proteins.

IGF2, IGF2R, Mitochondria, Neuroprotection, OxidativeStress, Synapse

P3-02

ERYTHROPOIETIN REDUCES CENTRAL PATHOLOGY AND COGNITIVE IMPAIRMENT IN A MURINE MODEL OF INTRAVENTRICULAR HEMORRHAGE IN THE PRETERM NEWBORN

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Intraventricular hemorrhage (IVH) is the most common intracranial condition of the preterm newborn (PTNB), affecting up to 15-20% of babies born before the 32nd week of gestation. Consequently, IVH is a major health problem associated with high personal and economical costs. IVH does not have a successful treatment and therefore, it remains crucial the search for new therapeutic alternatives. Previous studies have shown that erythropoietin (EPO) may contribute to maintain the integrity of the blood capillaries and the blood-brain barrier. EPO also favors neurogenesis and oligodendrogenesis, and it decreases brain inflammation and white matter damage in other central pathologies. However, to our knowledge, its effect on IVH of the PTNB has not been systematically studied. We have recently developed an animal model of IVH of the PTNB, by intraventricular administration of 0.3 IU of collagenase to 7 days of age (P7) CD1 mice. These animals show acute and long-term complications including brain atrophy, small vessel disease, inflammation, compromised neurogenesis and cognitive impairment. To analyze the neuroprotective role of EPO in our animal model, we have administered EPO ip for 3 consecutive days (P7-P9), immediately after collagenase lesions were performed. Animals have been assessed in the short (P14) and the long term (P70). We have observed that EPO treatment significantly reduces brain atrophy and ventricle enlargement, measured by cresyl violet staining, at P14 and P70. Neuronal simplification and small vessel bleeding, analyzed by Golgi-Cox and Prussian blue staining respectively, are also normalized in the cortex and the subventricular zone after EPO treatment. Moreover, learning and memory abilities, evaluated in the Morris water maze and the new object discrimination test, are preserved in IVH animals treated with EPO. Altogether, our data show that EPO treatment reduces central complications associated with HIV, including brain atrophy, neuronal simplification, small vessel bleeding or cognitive impairment, and support further studies with EPO as a feasible alternative to reduce central damage observed in the HIV of the RNPT. **Acknowledgements:** MG-A: Programa Estatal I+D+I Retos (BFU 2016-75038-R), financed by Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional (FEDER). Subvención para la financiación de la investigación y la innovación biomédica y en ciencias de la salud en el marco de la iniciativa territorial integrada 2014-2020 para la provincia de Cádiz. Consejería de Salud. Junta de Andalucía. Union Europea, financed by Fondo de Desarrollo Regional (FEDER) (PI-0008-2017).

Intraventricular hemorrhage, perterm newborn, collagenase, erythropoietin

P3-03

METHODOLOGY USED TO CARRY OUT A SYSTEMATIC REVIEW OF THE USE OF VIRTUAL REALITY IN THE REHABILITATION OF SPINAL CORD INJURIES

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Introduction: Although most studies on virtual reality show their effects on people with stroke, several recent studies have been conducted in the

field of spinal cord injury (Riva G. 2001; Kizony et al. 2005; Carlozzi et al. 2013). Objective: To analyze the information available on the effectiveness of VR in the treatment of disorders caused as a result of a spinal cord injury.

Methodology: A systematic review of clinical trials was conducted following the recommendations of the declaration Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for this type of study (Urrutia y Bonfill, 2010). The literature search was performed until April 2018 in the databases: CINAHL, Pubmed, Cochrane Central Register of Controlled Trials, PEDro, Scopus y Web of Science. The search strategy included different terms: Spinal Cord Injury (SCI): “spinal cord injury”, “spinal cord injuries”, “tetraplegia”, “quadriplegia” “paraplegia” and Virtual reality (VR): “virtual reality”, “virtual reality therapy” “virtual systems”, “augmented reality”. Depending on the information consulted, different search equations were formulated; always using the Boolean operator OR to locate all the articles containing the terms block Spinal Cord Injury; the same procedure was carried out with the Virtual Reality block, and finally, the two blocks were combined with the operator AND. Inclusion criteria: Persons with spinal cord injury. Clinical trials. Title and abstract in English. Publications in which spinal cord injuries are exclusively addressed and which use virtual reality systems. Exclusion criteria: Persons < 18 years. Persons with other neurological disorders. Publications in the form of abstracts. Selection of articles: The articles were selected by 3 reviewers following the inclusion and exclusion criteria, and eliminating duplicated articles. In addition to the searches made in the databases and electronic journals, the bibliographic references sections of the articles finally included in this work were also reviewed in order to locate additional studies that could fulfill the inclusion criteria.

Results: 640 articles, initially selected by their 242 abstracts, 93 of which were duplicated, leaving 149, which, on being selected in accordance with the search criteria, included 32 articles in the review, 8 of which correspond to randomized controlled trials (RCT).

Discussion/Conclusions: The results show the potential benefit of the virtual reality therapy in the treatment of people with spinal cord injury. It is worth nothing the reduced number of RCTs found in the present systematic review.

Registration: Prospero CRD42018093855 Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018093855

Spinal cord injury, virtual reality, rehabilitation

P3-04

BRIEF HIGH-FAT FEEDING IMPAIRS RETINAL DEGENERATION IN A MODEL OF RETINITIS PIGMENTOSA

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High-fat diet (HFD) feeding can induce hyperglycemia and metabolic disorders in rodents and has been associated with diabetic retinopathy. The aim of the current study was to investigate the effects of brief high-fat diet feeding in retinal degenerative diseases. Rd10, a mouse model of retinitis pigmentosa (RP), and C57BL/6J mice 19 days old were fed either a normal chow (5.5% fat kcal) or high-fat diet (61.6% fat kcal) for 2 or 3 weeks. The animals were handled according to current regulations for the use of laboratory animals (European Directive 2010/63/UE). At the end of the experimental period, the blood glucose curve was performed, the retinal function was evaluated by electroretinography and optomotor test, and the morphology of the retinas was assessed by vertical retinal cryostat sections stained either with hematoxylin or immunohistochemistry techniques. Brief HFD-fed animals gained

significantly more weight and developed reversible glucose intolerance, independently of the feeding period or strain. In rd10 mice, high-fat diet feeding produced faster deterioration of retinal responsiveness with decreased a- and b-waves amplitudes and lower visual acuity. This decrease was accompanied with higher reduction in the number of photoreceptor cells and shorter outer and inner segments. Moreover, a worsening of synaptic connectivity was observed with decreased density of presynaptic photoreceptor terminals and retraction of bipolar and horizontal cell dendrites. Brief high-fat feeding accelerates the spatiotemporal progression of retinal degenerative diseases such as RP. The results suggest that the consumption of high-fat diets by patients suffering from ocular neurodegenerative diseases could exacerbate the pathology.

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Neurodegeneration, hyperglycemia, metabolic disorders, obesity, rd10

P3-05

ASSOCIATION BETWEEN PHENOTYPE AND GENE EXPRESSION IN A MOUSE MODEL OF HUNTINGTON'S DISEASE MAINTAINED IN A PURE GENETIC BACKGROUND

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Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an aberrant expansion of CAG repeats in the exon 1 of the Huntingtin (HTT) gene. This disorder is characterized by motor impairment, cognitive deficits and psychiatric symptoms. Despite the inverse correlation between the number of repeats and age of disease onset (usually in the mid-adulthood), there exists a large variability in the manifestation of the symptomatology that justifies the search for genetic variants that modulate HD severity. However, and despite the retrieval of interesting candidates, the number and impact of such genetic modulators have been proven to be relatively low. Epigenetics comprises the changes in gene function, usually by altering gene expression, without altering the DNA sequence through covalent modifications of DNA and histones, providing a dynamic link between genetics and environment. We hypothesize that altered epigenetic patterns may provide meaningful insights about the phenotypical heterogeneity observed in this monogenic disorder. To explore this possibility, we used as a HD mouse model the transgenic R6/1 strain, which expresses the N-terminus of HTT gene bearing >110 glutamines. This strain can be maintained in a pure C57Bl/6 background to minimize the genetic component underlying the differential manifestation of pathological phenotypes between mutant mice. After assessing the early deficits of both motor and cognitive traits in a behavioural test battery (Morris water maze, novel object discrimination, rotarod, feet clasping, spontaneous activity), we classified the R6/1 mice in 'good', 'bad' and 'normal' performers according to their scores in the tests. From the same animals we examined different brain areas (prefrontal cortex, striatum, hippocampus, cerebellum) to conduct a transcriptional survey in order to correlate the degree of affection between gene expression and phenotype, prior to the study of epigenetic alterations. Whereas no clear pattern was observed in genes related with inflammation and gliosis, others belonging to a neuronal transcriptional signature in HD (e.g., Plk5, Penk, Itpka, Rin1) were in general more severely altered in the striatum of bad performers. In contrast, other brain areas (e.g., cerebellum) with a minor role in HD patients did not show such difference. This

study will provide novel insights to understand the molecular mechanisms involved in the differential manifestation of pathological traits in HD, to provide potential biomarkers, and to propose therapeutical targets for a personalized epigenetic-based treatment.

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Polyglutamine, motor, cognitive, transcription, heterogeneity

P3-06

DISTINCTIVE CHARACTERISTICS OF THE RETROGRADE RESPONSE TO AXONAL INJURY IN NEONATAL HYPOGLOSSAL MOTONEURONS

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Traumatic injury of a motor nerve at perinatal ages triggers a retrograde response resulting in degeneration and death of a high proportion of injured motoneurons. On the contrary, traumatic injury of a motor nerve in adulthood, a developmental stage in which damaged axons are able to regenerate and re-innervate their original target muscle, does not result in motoneuron death. Even though the retrograde response to axotomy has been well characterized in adult motoneurons, its components have not yet been fully defined in neonatal motoneurons. Several lines of evidence suggest that the degenerative process to which neonatal motoneurons are eventually committed has apoptotic character and is associated with excitotoxic mechanisms. Our preliminary hypothesis is that the distinctive characteristics of the retrograde response to axotomy in neonatal motoneurons could represent 'risk factors' that would render them more sensitive to the excitotoxic action of glutamate. By using the XIIth (hypoglossal) nerve crush injury model and electrophysiological and immunohistochemical techniques, this study provides a comprehensive description of the changes in intrinsic excitability, synaptic efficacy, excitation/inhibition balance, and protein expression patterns induced by axotomy in neonatal motoneurons of the hypoglossal nucleus (HMNs). Our data reveal profound alterations in both, passive and active membrane properties, which produce a dramatic increase in the intrinsic excitability of injured HMNs, even though they also tend to limit their ability to discharge at high frequency. Axonal injury also induces massive loss of afferent synaptic contacts affecting excitatory and inhibitory synaptic inputs to the same extent. Finally, immunohistochemical analysis shows upregulation of a glial reactivity marker (GFAP), downregulation of proteins associated with either regeneration (CGRP) or with maintenance of a functionally differentiated phenotype in HMNs (ChAT, NeuN and KCC2), and de novo expression of molecules that could play a key role in synapse withdrawal and neuronal death (nNOS and Caspase 3). In conclusion, our results show that the retrograde response to axotomy in neonatal HMNs differs qualitatively and quantitatively from that exhibited by adult HMNs. The peculiar components of this neonatal response might be implicated on regenerative processes, synaptic remodeling, and neuronal survival.

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Motoneurons, hypoglossal, axotomy, excitability, synapse, neurodegeneration

P3-07

AXONAL INJURY ALTERS THE EXPRESSION BALANCE AND SURFACE DISTRIBUTION OF NR2A- AND NR2B-CONTAINING NMDARs IN NEONATAL HYPOGLOSSAL MOTONEURONS

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In immature animals, motor nerve injury elicits elimination of afferent synaptic contacts and death of a high proportion of lesioned motoneurons. This type of injury has been frequently considered as an experimental model for the study of the pathogenic mechanisms that are inherent and common to many neurodegenerative diseases. An altered function of NMDA type glutamate receptors (NMDARs) seems to represent one of the earliest changes preceding neurodegeneration in most of these conditions. Our preliminary hypothesis is that the relative increment of the expression and function of extrasynaptic and/or NR2B-containing NMDARs could precede and be causally linked to synapse loss and activation of the neuronal death program in axotomized neonatal motoneurons. By using the hypoglossal nerve (XIIth) crushing injury model, we specifically aimed to determine whether axonal injury enhances the balance between extrasynaptic vs synaptic NMDARs and between NR2B- vs NR2A-containing NMDARs in neonatal hypoglossal motoneurons (HMNs). RT-PCR and western blot analysis revealed that the ratios of NR2B to NR2A RNAm and protein levels were reduced by more than 60% from postnatal day 3 (P3) to P8 in the hypoglossal nucleus (HN). Interestingly, XIIth nerve crushing at P3 apparently occluded this developmental change, thereby preserving an enhanced NR2B/NR2A ratio in the lesioned HN at P8. Accordingly, immunofluorescence labelling of brainstem sections from lesioned animals showed that the staining intensity for NR2B markedly increased in HMN cell bodies on the injury side relative to the intact HN, whereas NR2A immunoreactivity dramatically declined. Finally, to analyze membrane expression of NR2B and NR2A we performed whole-cell patch clamp recordings of HMNs using subtype-selective antagonists. The combined electrophysiological analysis further shows an overall reduction in functional expression of NR2A subunits along with relative increases in both, contribution of NR2B to the synaptic and extrasynaptic NMDAR-mediated currents and the magnitude of extrasynaptic NMDAR-mediated currents in lesioned HMNs. We conclude that axonal injury differentially regulates surface expression of synaptic and extrasynaptic NMDARs as well as of NR2A and NR2B subunits in neonatal HMNs, which results in an elevated NR2B/NR2A ratio at the synaptic and extrasynaptic membranes. The relative increment in membrane expression of NR2B-containing NMDARs in axotomized HMNs could promote the emergence of intracellular signaling pathways involved in the dismantling of afferent synaptic contacts and activation of the neuronal death program.

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Motoneuron, axotomy, NMDA, NR2, neurodegeneration, synapse

P3-08

SCREENING OF PHORBOL ESTERS FROM EUPHORBIA RESINIFERA WITH NEURAL PROGENITOR CELL PROMOTION ACTIVITY

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12-Deoxyphorbols (1-3) from *Euphorbia resinifera* have been described by our research group as non-tumorigenic promoters of neural progenitor cells (NPCs) expansion.[1] The activity of this class of compounds is correlated with the modulation of protein kinase C (PKC).[1] Phorbol esters modulation of PKCs involves binding with C1 protein domain and translocation of the complex to membrane, through hydrogen bond interactions of oxygenated functional groups at C-3, C-4 and C-20 on phorbol derivatives, with specific protein residues.[2] Translocation of the complex phorbol/PKC within the cell depends of the nature of esters chains, for instance, at C-12 and C-13.[3] and this, on the other hand, has an influence on the nature of the biological activity. Further work on this class of compounds requires discovery of novel natural phorbol derivatives for structure-activity relationship studies. LC-MS profiling of diterpenes of *Euphorbia* spp. has been described previously and has assisted in the analysis of complex extracts.[4] In this communication, we describe an UPLC-HRMS assisted screening of the extract of the latex from *E. resinifera*, with the help of suitable synthetic models, aiming at the discovery of phorbol esters with different functionalization patterns to those previously described.[1]. Evaluation of the neural progenitor cell promotion activity is described. [1] N. Geribaldi-Doldán et al. *Int. J. Neuropsychopharmacol.* 2016, 19, 1-14; doi: 10.1093/ijnp/pyv085. [2] J. Das and G. M. Rahman *Chem. Rev.* 2014, 114, 12108-12131. [3] Q. J. Wang et al. *J. Biol. Chem.* 2000, 275, 12136-12146. [4] Nothias-Scaglia, L.-F. et al. *J. Chromatogr. A* 2015, 1422, 128–139.

UPLC-HRMS, *Euphorbia-resinifera*, phorbol-ester, neural-progenitor-cell

P3-09

PURINERGIC SIGNALING REGULATES CELL FATE DETERMINATION IN POSTNATAL SUBVENTRICULAR ZONE NEURAL PROGENITOR CELLS

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The subventricular zone (SVZ) of mammalian brains contains neural progenitor cells (NPCs) from which new neurons are formed throughout life. Specifically, SVZ NPCs produce neuroblasts that migrate towards the olfactory bulb to finally differentiate to granule and periglomerular interneurons. Proliferation and differentiation of NPCs within this neurogenic niche depend on cell-cell communication conducted by diffusible factors or by direct physical cell-cell contacts. Signaling mediated by ATP and other purine compounds intervenes on the proliferation of SVZ NPCs in vitro and in vivo, with variable effects according to the age and the pathological condition. However, the role of purinergic signaling in NPC differentiation has not been fully investigated. In this study we have analysed the effect of pharmacological blockade of purinergic receptors in the differentiation pattern of SVZ NPCs isolated from 7-day postnatal rats. NPCs were grown as neurospheres and they were induced to differentiate on an adhesive substrate in the absence of mitogens. Adhered

cultures were treated with suramin, a blocker of P2 purinergic receptors (200 μ M), or with vehicle. After 48h, cultures were fixed and processed for immunohistochemistry to analyse differentiation to astrocytes, oligodendrocyte precursors and neurons. Treatment with suramin induced severe morphological changes in the NPCs that showed a significant reduction in the length of their cellular processes. Cell viability was also affected by the treatment since the number of surviving cells was reduced in cultures treated with suramin ($p < 0,05$). Most cells ($91,5 \pm 2,1$ %) from suramin-treated cultures retained the expression of the neural precursor marker nestin whereas this percentage was significantly lower in control cultures ($65,4 \pm 5,3$ %). In addition, the percentages of astrocytes and of oligodendrocyte precursors were reduced by half as a result of the treatment with suramin. No significant effects were observed in the neuronal differentiation pattern. Our results show that ATP is needed to initiate differentiation of postnatal SVZ NPCs since when its signaling is lacking, surviving cells retain the expression of nestin. In addition ATP induces differentiation towards glial phenotypes. Therefore, signaling mediated by ATP might be important in the mechanisms involved in the control of NPC differentiation in the SVZ neurogenic niche.

ATP, neural stem cell, differentiation, neurospheres

P3-10

LIPID PEROXIDATION INDUCED BY CUMENE HYDROPEROXIDE ABOLISHES A TONIC GABAA RECEPTOR-MEDIATED CURRENT IN RAT PRIMARY MOTOR CORTEX NEURONS

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Introduction: In Amyotrophic Lateral Sclerosis (ALS), layer V pyramidal motor cortex neurons that regulate voluntary control of the motor output, are selectively degenerated. It has been proposed that this degeneration may be the result of cortical hyperexcitability due to the interruption of cortical inhibition. However, the mechanisms underlying this hyperexcitability are still unknown. We have previously shown that lipid peroxidation induced by cumene hydroperoxide (CH) produces synaptic depression of this pool of motor neurons characterized by a decrease in the frequency and the amplitude of postsynaptic currents. These decreases are greater and faster on the GABAergic transmission1 and could underlie the increased excitability. However, the hiperexcitability may be not only due to the block of phasic inhibitory postsynaptic current (IPSC) but also to the block of a tonic inhibitory current, if it present.

Methodology: To determine if the motor neurons have a tonic GABAA receptor-mediated current and how it is affected by oxidative stress, whole-cell patch-clamp recordings in voltage clamp mode were obtained from layer V pyramidal neurons of the motor cortex in rat brain slices. Tonic GABAA receptor-mediated currents were revealed by blocking the GABAA receptor with the antagonist gabazine (20 μ M) in the presence of CNQX (50 μ M) and APV (20 μ M) to block the glutamatergic currents ($n = 10$). Lipid peroxidation was induced by the administration of CH (10 μ M).

Results: In presence of gabazine, the spontaneous IPSC were blocked, and a tonic current depending on GABAA receptors was unmasked (51.0 ± 10.7 pA). When we repeat the experiment replacing gabazine with CH we observe that a similar current was blocked (50.6 ± 13.9 pA). Finally, adding CH to perfusion after gabazine exposition did not have any effect on the tonic current ($48.2 + 12.3$ pA vs. $51.0 \pm 10, 7$ pA). This results course in parallel with previous results found in current clamp mode, in which the application of gabazine or CH increased membrane resistance1, but any additional effect were found if they are added consecutively.

Conclusion: Pyramidal neurons of the motor cortex showed a tonic GABAA receptor-mediated current that was abolished by CH. This mechanism could

contribute to the hyperexcitability found after the oxidant administration and characterized by increased resistance and decreased rheobase. It could be propose that lipid peroxidation, via alteration of cortical inhibition, contributes to the causes underlying ALS. 1. Pardillo-Diaz et al., 2017. *Mol. Cell. Neurosci.* 82, 204–217.

Lipid peroxidation, hyperexcitability, tonic current

P3-11

NEURONAL DIFFERENTIATION OF RATS ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Introduction: Mesenchymal stem cells (MSCs) derived from adipose tissue (ADSCs) have the ability to transdifferentiate into other lineages besides their own. Most studies on neuronal differentiation of MSCs have focused on a morphological and immunophenotypical characterization while few information are available for electrophysiological changes of neuronal differentiation. The aim of the present work was to develop a protocol to differentiate ADSCs up to cells with neuronal phenotype and to evaluate the moment in which they become mature neurons showing electrophysiological features.

Methodology: Mesenchymal cells were extracted from rat adipose tissue and were first subjected to a multipotentiality test to verify the cell type. Then, these cells were induced to neurospheres and from them differentiated to neurons. The cells were labeled with antibodies against Nestin and microtubule-associated protein 2 (MAP2) which are markers of neuronal stem cells (NSCs) and mature neurons, respectively. Whole cell patch-clamp recording were performed to evaluate the electrophysiological properties during neuronal differentiation. Membrane potential and passive properties were measured in current current-clamp mode and voltage-gated currents in voltage-clamp mode.

Results: The immunocytochemical labeling with Nestin and MAP2 showed that from day 18 of culture the cells reach the neuronal phenotype. This early differentiated neurons displayed elongated shape with protrusion of two or three cellular processes at day 18. After 25 days of cultures, differentiated cells showed a characteristic neuronal morphology with contracted cytoplasm, condensed nucleus and protrusion of three-six cellular processes that displayed apparent synaptic connections with nearby neurons. These neurons showed a membrane potential of about -60 mV, more negative than early differentiated cells (about -30 mV). Lower values of membrane capacitance were also observed for differentiated cells respected to the others. Differentiated neurons also exhibited prominent inward and outward currents. These currents were characterized as voltage dependent sodium and potassium.

Conclusion: Cells from day 18 of culture develop morphology and neuronal phenotype, presenting also Na^+ and K^+ dependent-voltage currents like neurons. In view of these results neuronal differentiation of ADSCs presents a great potential in the treatment of neurodegenerative diseases.

Mesenchymal stem cells, neuronal differentiation

P3-12

PKC ACTIVATION BY INTRANASAL ADMINISTRATION OF NON-TUMOR PROMOTING 12-DEOXYPHORBOLS INDUCES NEUROGENESIS FACILITATING MEMORY AND LEARNING

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Pharmacological strategies aimed to facilitate neuronal renewal in the adult brain, by promoting endogenous neurogenesis, constitute promising therapeutic options for pathological or traumatic brain lesions. We have previously shown that non-tumour-promoting PKC-activating compounds (12-deoxyphorbols) promote adult neural progenitor cell (NPC) proliferation in vitro and in vivo enhancing the endogenous neurogenic response of the brain. Particularly, intracerebroventricular injections of 12-deoxyphorbols such as prostratin and ER272, isolated from *E. resinifera*, induce the proliferation of NPC within the dentate gyrus of the hippocampus and the subventricular zone on in vivo facilitating the generation of new neurons. These diterpenes exerted their effects through a mechanism that involved the activation of protein kinase C (PKC), therefore, 12-deoxyphorbols such as ER272 might be of use in the development of treatments aimed to facilitate neuronal replacement. However, it becomes necessary to find a non-invasive administration method to deliver this compound in the adult brain overcoming the blood brain barrier. Thus, we have tested in here the effect of intranasal administration of 12-deoxyphorbols during 3, 7 and 28 days. Intranasal administration of ER272 during 3, 7 and 28 days increases NPC proliferation in the SVZ and in the DG compared with the control. In addition intranasal administration of ER272 for 28 days improves memory and learning tasks. These results suggest that intranasal administration is a good non-invasive method of administration of 12-deoxyphorbols, which can be used to develop pharmacological drugs aimed to promote neuronal replacement in pathological or traumatic lesions.

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Neurogenesis, stem cells, PKC, brain injury

P3-13

INHIBITION OF PROTEIN KINASE C PROMOTES NEUROGENESIS IN INJURED BRAIN CORTEX

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Acute or chronic injuries cause damage in the adult brain producing neuronal death. Neural stem cells (NSC) are activated inside neurogenic niches in response to those injuries producing neuroblasts, which attempt to migrate towards the injured area, but this response does not contribute to the generation of new neurons within the damaged tissues. Neurogenesis in lesions is very limited and there is no effective treatment to generate neurons that compensate or ameliorate neuronal loss. Injuries constitute a gliogenic/non-neurogenic niche in which neuronal production is impaired since signaling molecules within the injury released by the inflammatory response act on intracellular pathways that prevent the generation of neurons. Therefore, the search for signaling molecules to target in order to prevent this phenomenon has become a key step at designing strategies to stimulate the neuronal replacement in injured brain tissue. Kinases of the Protein kinase C (PKC) family are intracellular kinases that participate in multiple signaling pathways involved in neurogenesis. Activation of PKC isotypes such as PKC β and

PKC α stimulate neural progenitor cells (NPC) proliferation leading to the generation of neuroblasts and neurons. We have found in here that PKC inhibition *in vitro* in NPC cultures leads to neural progenitor cell differentiation towards a neuronal fate facilitating the generation of neurons, this effect is mediated by the inhibition of PKC ϵ . We also demonstrate that PKC ϵ is expressed in cortical injuries although it is not the most abundant. Inhibition of PKC activity *in vivo* leads to neuronal differentiation in brain injuries and to a large increase in the generation of neurons in brain injuries. Our findings suggest that PKC ϵ might be used as a target to design pharmacological drugs aimed to regenerate brain lesions. Notes: Care and handling of animals were performed according to the Guidelines of the European Union Council (2010/63/EU), and following the Spanish regulations (65/2012 and RD53/2013) for the use of laboratory animals. **Acknowledgements:** This work was supported by the Spanish Consejería de Innovación, Ciencia y Empleo, Junta de Andalucía (grant numbers P10CTS6639), and by Ministerio de Economía y Competitividad (grant numbers BFU2015-68652-R, and BFU2016-75038-R MINECO/FEDER).

Neurogenesis. Stem Cells. PKCs. Brain Injury

P3-14

INHIBITION OF ADAM17/TGF-ALPHA/EGFR PATHWAY PROMOTES NEUROGENESIS WITHIN TRAUMATIC BRAIN INJURIES IN MICE

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Activation of neural stem cells (NSC) and differentiation into neuroblasts occurs in response to traumatic brain injuries. Neuroblast generated in neurogenic regions such as the dentate gyrus (DG) of the hippocampus or the subventricular zone (SVZ) alter their migration patterns and migrate towards the injured region in an attempt to repair the affected areas. However, the local environment of the injured area does not facilitate the generation of new neurons. Instead, the environment facilitates gliogenesis. Previous results from our laboratory have shown the role of the sequential activation of ADAM17/TGF- α /EGFR pathway in the generation of the gliogenic environment within brain lesions. We show in here that inhibition of this signaling cascade by a general metalloprotease inhibitor allows the generation of neuroblast within injured areas. These new neuroblast have either migrated towards the injury from neurogenic regions or they have been generated from NSC activated at the site of injury. In order to specifically inhibit the metalloprotease ADAM17, we have created a lentiviral vector containing the pro-domain of ADAM17 (ADAM17-Pro). The transfection of ADAM17-Pro into neural progenitor cell (NPC) derived from the SVZ showed a 2-fold increase in neuronal differentiation. Consistent with the above, the injection of this lentivirus in injured areas of the brain increased the number of neuroblasts in the damaged regions 14 days after injury. In addition, those neuroblasts produced neurons a large number of cholinergic and GABAergic neurons 28 days after injury, revealing the antagonistic role of ADAM17 in the differentiation of neural stem cells.

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ADAM17, TGF-ALPHA, EGFR, neurogenesis, brain injury

P3-15

ROLE OF LIRAGLUTIDE ON METABOLIC CONTROL AND COGNITIVE IMPAIRMENT IN A MURINE MODEL OF TYPE 2 DIABETES AND ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common cause of dementia, is characterized by a progressive deterioration of cerebral functions, affecting the decision-making capacity. AD can only be histologically diagnosed postmortem by the presence of senile plaques (SP), neurofibrillary tangles and neuronal and synaptic loss. Although the main risk factor for this pathology is age, multiple studies associate this pathology with type 2 diabetes mellitus (T2D) as an important risk factor. Despite the evidence supporting the AD-T2D relationship, the basis are not completely elucidated. To further contribute to study the role of T2D in AD we have developed a mixed model of AD and DM2, the APP/PS1 \times db/db mouse AD-T2D mice were produced by cross-breeding APP^{swe}/PS1^{de9} with db/db mice. It is important to consider that AD does not have an effective treatment, making necessary to search new therapeutic alternatives for prevent, delay or treat AD. Although with evident limitations, T2D has different therapeutic options. In order to explore the role of glucagon-like peptide 1 (GLP-1) receptor agonist's liraglutide (LRGT) we have administered as alternative to slow down or retard the metabolic and cognitive complications in a mixed model of T2D and AD. Animals received LRGT (100-500 μ g/kg/day, s.c.) or PBS (control group) every day from 6 to 26 weeks of age. Body weight, glucose and insulin levels are determined before treatment and every 4 weeks until sacrifice. We also assess the role of LRGT on cognition two weeks before the sacrifice. For this purpose, we use the Morris water maze test to assess spatial memory and the new object discrimination test to analyse episodic memory. We expect LRGT to control metabolic alterations in db/db and APP/PS1 \times db/db mice by reducing glucose levels and maintaining insulin levels in the long term. In addition, metabolic control with LRGT treatment may also contribute to improve learning and memory impairment in APP/PS1 \times db/db mice. In addition, we believe Altogether, LRGT may provide be an alternative to control or slow down central complications associated to T2D and AD.

Alzheimer's disease, diabetes, liraglutide

P3-16

MODULATIONS ON COGNITIVE PERFORMANCE AND ELECTROPHYSIOLOGICAL ACTIVITY BY DEEP BRAIN STIMULATION OF HUMAN NUCLEUS ACCUMBENS IN OCD PATIENTS

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Deep brain stimulation (DBS) has emerged as a safe, effective, and reversible treatment for a number of neurological and psychiatric disorders. Recently, DBS has been also introduced as a neurosurgical treatment for refractory Obsessive-compulsive Disorder (OCD) where the nucleus accumbens (Nacc) has prevailed as the preferred target. In early clinical trials, fifty percent of OCD patients treated with DBS showed significant improvements in symptoms and global functioning. OCD is one of the most disabling of all psychiatric illnesses and at least 10% of are refractory to psychopharmacological treatment. Core symptoms of OCD are recurrent and intrusive thoughts and repetitive behaviours, however is also associated with significant cognitive impairment, including decision making processes, reinforcement learning and response inhibition actions or behaviours. The aim of this study was to explore the underlying electrophysiological mechanisms characteristic of NAcc-DBS using scalp EEG during a sustained attention cognitive control task performance, to examine context processing and goal maintenance. In order to contrast the electrophysiological correlates of the task related activity as such, with the activity during DBS. Eight patients undergoing NAcc-DBS for treatment of refractory OCD performed a cognitive task (an AX-CPT paradigm with equiprobable Go/NoGo conditions) during the recording of scalp event-related potentials (ERP) and simultaneous DBS. NAcc-DBS resulted in increased intra-individual reaction time variability in the ON state relative to the OFF state. Moreover, NAcc stimulation revealed that preparing to respond produced a more focally distributed frontocentral negativity (contingent negative variation; CNV). A significant increase in P3 amplitude over the parieto-occipital regions following the presentation of Go-trials and an increased N2 amplitude over frontal and parietal regions during NoGo trials were also found. Brain source reconstruction of P3 elicited by Go-trials resulted both in a significant and more widely distributed parietal pattern of cortical activations and in reduced right prefrontal activations during NAcc-DBS "ON" relative to "OFF". Our results show that DBS of the NAcc in OCD patients appears to alter neuronal activity underlying sustained speeded responses and complex information processing, which is likely caused by a dopaminergic-mediated "regulation" of the critical interactions of a frontal-striatal-thalamic-cortical network.

Nac, DBS, OCD, ERP, EEG

P3-17

DIABETES MELLITUS, DEPRESSION AND THEIR CONTRIBUTION TO ALZHEIMER'S DISEASE. A RETROSPECTIVE CASE-CONTROL STUDY

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Dementia is defined as an acquired cognitive skills deterioration that gradually affects a person's ability to perform everyday activities. The most common cause of dementia in old age is Alzheimer's disease (AD). AD is an important problem in public health worldwide. Although no disease-modifying treatment for any common dementia is available, current research focuses on

prevention of risk factors considered modifiable which is expected to delay the onset and decrease incidence or prevalence of age-specific dementia. Among all risk factors that have been studied for AD, those with more evidence are diabetes mellitus (DM) and depression. They are also relevant because of their high prevalence in society and because they have proved to interact with each other. Therefore, the aim of this work is to determine the presence of DM and depression at base-line and whether are more frequent in the incident cases of AD than in a group of similar age no cognitively affected. A retrospective analytic observational case-control study has taken place throughout eight-year (April 2008 to March 2016) with ambulatory patients from the Neurology Service at the Hospital Universitario Puerta del Mar. Attending for the first time consultation, participants were divided into two groups: 577 AD cases and 467 control patients ≥ 56 years old without cognitive symptoms. Data source was the digital medical records. Age, sex, and DM and depression presents at base-line were collected and reviewed. Female sex is more frequent in cases (75.39%) than in controls (62.35%) ($P=0.0000$; OR 1.83 [1.40-2.39]). Average onset age is higher in cases (77.90 ± 6.88 sd) than in controls (71.46 ± 8.05) ($P=0.0000$). DM is not more frequent in cases (27.08%) than controls (32.12%) ($P=0.78$ [0.60-1.02]). Depression is statistically significant among AD group (22.05%) than in the control group (15.20%) ($P=0.0065$; OR 1.58 [1.14-2.17]). DM2 and depression association is not more frequent among cases than controls (4.71%) ($P=0.6831$; OR 1.18 [0.65-2.15]). As a result, depression should be considered a significant factor in the prevention of AD.

Alzheimer's disease, diabetes mellitus, depression

P3-18

BASELINE CHARACTERISTICS OF FRONTOTEMPORAL DEMENTIA AND LEWY BODY DEMENTIA. A RETROSPECTIVE STUDY

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Neurodegenerative diseases result of complex interactions of different influences such as genetic susceptibility, psychosocial factors, biological factors, and environmental exposures. Often they have overlapping features, and are heterogeneous in their clinical presentation, coexisting both typical pathological changes as a variety of the circumstances above indicated. In the case of Dementia with Lewy bodies (LBD) and Frontotemporal dementia (FTD), the most frequent types of neurodegenerative disorders after Alzheimer's disease (AD) and Vascular Dementia, where the differential diagnosis is still a complex clinical process. Frequently these pathologies are concomitant (mixed dementia) and show an additive and synergistic contribution to cognitive impairment. Not many epidemiological studies about FTD and LBD have been conducted so the purpose of this work is to know more about the background of these dementias in order to understand the diagnosis, evolution and prevention. A retrospective study has been conducted throughout eight-year (April 2008 to March 2016) with ambulatory patients from the Neurology Service at the Hospital Universitario Puerta del Mar. Data source was the digital medical record. Attending for the first time consultation, different variables have been recollected such as demographic parameters, psychosocial antecedents, metabolic syndrome, other comorbidities, and evolution of this dementias to Alzheimer. From a total of 1108 patients diagnosed of dementia, 24 patients with LBD and 31 with FTD were identified, representing respectively 2.76% and 2.14% of dementia, 45.16% of

which were women. The average age at onset of LBD was 78.62 ± 3.5 ; onset average age of FTD was earlier, 68.67 ± 4 for FTD, but higher than the literature establishes (56 years). Not significant differences were found in terms of comorbidity. However, frequency of diabetes mellitus was greater in DLB patients (62.50%) than DFT (35.48%) or patients from our community (27.08% in AD; 32.12% in neurological patients similar aged). Only the patients of FTD (22.58%) evolved to AD.

FTD, LBD, risk factor, comorbidity

P3-19

EXPRESSION PATTERNS OF PROTEIN KINASE C ISOTYPES IN NEUROGENIC REGIONS AFTER BRAIN INJURY

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Background: The protein kinase C (PKC) family of serine/threonine kinases exhibit distinct patterns of tissue expression and have different functions in a variety of physiological and pathological brain processes. Particularly, PKC activation promotes neurogenesis in neurogenic regions of the adult brain. However, little is known about the role of PKC on neurogenesis in brain injuries. Therefore, in an attempt to understand the role of specific PKC isotypes in brain lesions we have analyzed the expression pattern of these kinases in the perilesional area and in the adjacent neurogenic niches: SVZ and DG.

Methods: We have performed controlled unilateral mechanical injuries in the primary motor cortex of mice. Using rt-PCR, we have analyzed patterns of expression of PKC isotypes in the SVZ, DG and at the site of injury, at 3, 14 and 28 days post-injury (dpi) comparing ipsilateral and contralateral hemisphere, in relation to control animals.

Results: Increased expression of different PKC isotypes was noted in response to injury in the cerebral cortex. In acute phase of lesion (3dpi), isotypes involved in cell proliferation such as PKC δ or PKC η , or in the activation of transcription factors involved in cell survival and inflammation, increased within the perilesional area and within more distant neurogenic niches. Expression of PKCs induced during the acute phase of the injury were normalized over time and no significant differences were observed 14 or 28 dpi relative to control animals in all the cerebral zones analyzed.

Conclusion: An acute response to the lesion can be observed both locally and in distant neurogenic niches, inducing the expression of PKCs involved in proliferation, inflammation and cell survival, probably in an attempt to protect the tissue from the damage. This response is diluted over time, restoring the expression patterns of the different PKC isotypes.

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PKC, neurogenesis, SVZ, cortex injury

P3-20

LOCALIZATION OF AQP9 IN MURINE BRAIN AND EFFECT OF AGING OVER ITS EXPRESSION PATTERN

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AQPs are a family of small integral membrane proteins that facilitate the transport of water across the cell membrane and, in some cases, few other small molecules. Nowadays, thirteen members of this family are known and expressed in many different tissues in mammals. In the brain, AQP4 is the most abundant of these proteins, but AQP1 and AQP9 are also expressed. We have recently demonstrated that hypoxia and aging act together to produce hydrocephalus in mice by a process that depends on AQP4. In the present work we analyze the expression levels and the cellular localization of AQP9 in young (2-3 months age) and aged (>14 months) mice. Choroid plexus, striatum, cortex and ependymal tissue were analyzed for mRNA levels by RT-qPCR; whereas localization of AQP9 protein was analyzed by immunofluorescence microscopy. Immunofluorescence of the astrocytic marker GFAP was also analyzed. Our data prove that AQP9 is expressed in astrocytes lining the ependymal membrane that border the ventricles and on the ependymal cells itself. Also signal was observed on the endothelium of blood vessels of pial cortex, and over neurons located in the inner cortex. The aging of animals produces an increase of AQP9 levels especially in the inner layers (III-VI) of the cortex that is not detected in the ependymal tissue. Also increase of GFAP signal was observed in the aged animals, indicating probably a gliosis process. We hypothesized that AQP9 contributes together with AQP1 and AQP4 to the CSF formation and movement across brain compartments. Moreover, the changes observed by aging on AQP9 would participate to the physiological events that along live span would contribute to development of chronic adult hydrocephalus.

Astrocytes, Aquaporins, AQP9, edema, hydrocephalus, aging

P3-21

ATP13A2 LEVELS ARE ELEVATED IN THE CEREBROSPINAL FLUID, SERUM AND/OR SALIVA OF PATIENTS WITH PARKINSON DISEASE

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Loss of function of ATP13A2, is causative of Kufor-Rakeb syndrome, a genetic form of Parkinson's disease (PD), as well as juvenile and young-onset forms of idiopathic PD. ATP13A2, an ubiquitous protein, possesses an ATPase domain that participates in mitochondrial and lysosomal ATPase activity. The objectives were: a) to measure ATP13A2 content in cerebrospinal fluid (CSF), serum and saliva of patients with PD; and 2) to analyze clinical correlations and ATPase disturbances. CSF, saliva and serum were obtained from PD patients and healthy controls. Informed consent forms under a protocol approved by Valme Hospital of Sevilla internal ethics and scientific boards were obtained from all the subjects. ELISA methods were used. Motor and global UPDR scales were evaluated. The findings revealed that ATP13A2 content was found to be elevated in some of these fluids, relative to controls. Thus mean ATP13A2 content was significantly enhanced in CSF ($p < 0.01$ vs. controls, Student), serum ($p < 0.05$) and/or saliva of patients ($p < 0.01$). ATPase activity of the

CSF was found to be significantly reduced in patients ($p < 0.02$), and it was negatively correlated to ATP13A2 levels ($p < 0.01$, Pearson). Furthermore, CSF ATP13A2 levels were found to be negatively correlated with motor deterioration as evaluated through UPDRS ($p < 0.05$). In conclusion, ATP13A2 is over-expressed in CSF, serum or saliva of sporadic PD patients. This change is related to reduced ATPase activity in the CSF, although ATP13A2 elevation in the CSF could be a defensive mechanism against motor deterioration.

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ATP13A2, Parkinson, cerebrospinal fluid, saliva

P3-22

ANALYSIS OF DOPAMINOTROPHIC AND INFLAMMATORY FACTORS, AS WELL AS ANTIOXIDANT EFFICACY IN THE CEREBROSPINAL FLUID OF THE ELDERLY

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Dopamine midbrain neuronal loss and neuroinflammation are two phenomena that are associated with brain senescence. Neurotrophic factor changes and oxidative stress could subserve these phenomena. Aging-related brain changes can be well monitored through the cerebrospinal fluid (CSF). The objective was to analyze neurotrophic and oxidative parameters that could be related to dopamine midbrain loss or neuroinflammation in the CSF of elderly subjects: 1) levels of the dopaminotrophic factors BDNF, GDNF, persephin, and neurturin, 2) levels of the proinflammatory factors TGF β 1 and TGF β 2; 3) activity of main antioxidant enzymes (catalases, glutathione-peroxidase, glutathione-reductase, glutathione-S-transferases, peroxiredoxins, and superoxide-dismutases), 4) ferritin content, antioxidant protein which reduces reactive free iron, and 5) antioxidant potential of the cerebrospinal fluid. ELISA and PAO tests were used. Subjects were also evaluated clinically. The findings indicate that normal elderly CSF is devoid of changes in either dopaminotrophic or proinflammatory factors. The antioxidant efficacy is slightly altered with normal aging, except for a reduction of glutathione-S-transferase activity in people older than 74 years ($p < 0.05$). However old people with mild cognitive impairment show reduced BDNF levels, and stronger signs of oxidative stress such as low antioxidant potential and reduced glutathione-S-transferase activity ($p < 0.05$). The present study demonstrates that, in CSF of normal senescence, dopaminotrophic factors and proinflammatory TGF-family ligands are not affected, and antioxidant efficacy is slightly reduced. CSF of elderly subjects with mild cognitive impairment shows more oxidative and trophic changes characterized by reduction of BDNF content, glutathione-S-transferase activity, and antioxidant potential. Supported to EFE by Junta de Andalucía (BIO127) and Sociedad Andaluza de Neurología (SUBAIA2015/006; SAN).

Aging, inflammation, oxidation, cerebrospinal fluid

P3-23

ARE EXOSOMES THE ONLY MICROVESICLES ABLE TO PROVIDE INFORMATION ON THE PHYSIOPATHOLOGY OF DRUG-INDUCED ACUTE KIDNEY INJURY?

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Introduction and aims: Exosomes are small membranous vesicles 30–150 nm in diameter released by many cell types to different biological fluids, including plasma and urine. At first, it was thought that exosomes simply served as 'garbage bags' for cells to eliminate unwanted components. But thanks to recent findings, we know that they play a relevant role in a wide variety of functions. Their composition, which consists of molecular constituents from their original cells, which include proteins, lipids, mRNA and microRNA (miRNA), make them promising biomarkers of certain pathologies, and thus they are a potential diagnostic tool. For this reason, we study these microvesicles in a experimental model of drug (cisplatin, gentamicin)-induced acute kidney injury (AKI). **Methods:** Male Wistar rats were treated with a single dose of cisplatin (5mg/kg), six doses of gentamicin (150mg/kg) (both AKI groups) or saline (control group). Urine and blood samples were collected in: Day 4 in the cisplatin group and day 6 in the gentamicin grupo (AKI development, serum creatinine (sCr) >2). Urinary microvesicles were isolated using a commercial kit for their precipitation and purification. We used electronic microscopy and the Nanosight system, which provided the size and concentration of particles per sample.

Results: Rats receiving cisplatin and gentamicin developed AKI (with increased sCr). The concentration of particles/ml in all experimental groups was similar. However, the size pattern of the isolated microvesicles in the experimental groups was different. In urine from the control group the size of microvesicle population was homogeneous, whereas it was heterogeneous in that of the AKI groups.

Conclusions: Summarizing, depending on the drug used the size of urinary microvesicles is different from those of the control group, and thus, a potential biomarker might be other nanoparticles that have not yet been identified or defined in the literature and that could provide more information about the physiopathology of AKI.

AKI, exosomes, microvesicles, cisplatin, gentamicin

P3-24

MELATONIN INDUCES REACTIVE OXYGEN SPECIES GENERATION AND REDUCES VIABILITY OF HUMAN PANCREATIC STELLATE CELLS

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In the recent years the interest on the role of pancreatic stellate cells (PSC) in the physiology and the pathophysiology of the pancreas has increased. Under normal conditions PSC remain in a quiescent state, but become activated in disease. It has been highlighted that activated PSC are responsible for the progressive fibrosis and for the accumulation of extracellular matrix that occurs in chronic pancreatitis and in pancreatic cancer. Therefore, an interesting approach for the treatment of pancreatic illness is to gain understanding of the mechanisms that are involved in the

regulation of PSC physiology. Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine produced in many organs within the body. It is primarily involved in circadian regulation of physiological functions. In addition, it possesses free radical scavenging properties and, therefore, it has been suggested that the indole exerts protective actions. However, studies have found that melatonin exerted pro-oxidant actions in tumor and non-tumor cells; therefore, melatonin could function as a potential regulator in disease. In this study we have examined the effects of pharmacological concentrations of melatonin (1 μ M - 1 mM) on human pancreatic stellate cells (HPSC). Primary cultures of HPSC were prepared by collagenase digestion of samples of human pancreas. The expression of cell type specific markers and of melatonin receptors was analyzed by western blot analysis. Fluorimetric analysis of fura-2-loaded cells was used to follow changes in intracellular free Ca^{2+} concentration. Production of reactive oxygen species (ROS) was monitored following CM-H2DCFDA and MitoSOXTM Red-derived fluorescence. Finally, cell viability was studied using AlamarBlue® test. Our results show that cultured cells expressed markers typical of stellate cells. However, cell membrane-receptors for melatonin could not be detected. Thapsigargin or melatonin induced changes in intracellular free- Ca^{2+} concentration. The indoleamine evoked a concentration-dependent increase in ROS production in the mitochondria and in the cytosol. Finally, melatonin decreased HPSC viability in a time and concentration-dependent manner. In conclusion, our findings show that pharmacological concentrations of melatonin might create pro-oxidative conditions within HPSC that modulate their proliferation. The actions of melatonin might not involve specific plasma membrane receptors. Our observations point towards a role of the indoleamine in the modulation of pancreatic fibrosis. Funding for this study was partly provided by Ministerio de Economía y Competitividad (BFU2016-79259-R) and Junta de Extremadura-FEDER (IB16006).

Melatonin, calcium, ROS, cell viability, HPSC

P3-25

MELATONIN MODULATES DYSREGULATED CIRCADIAN CLOCKS IN A MICE MODEL OF DIETHYLNITROSAMINE-INDUCED HEPATOCELLULAR CARCINOMA

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Disruption of circadian rhythms, which are regulated by clock genes, results in pathological processes including hepatocellular carcinoma (HCC). The main complex of clock genes is the heterodimer brain and muscle *amt*-like protein-1/circadian locomotor output cycles protein *kaput* (BMAL1/CLOCK), which regulates the expression of the transcriptional repressors cryptochrome (CRY) and period (PER). The modulation of clock genes has been suggested as cancer therapeutic target. Melatonin exhibits oncostatic features, but it is unknown if these effects are mediated by the regulation of circadian rhythms. Mice in treatment groups received diethylnitrosamine (DEN) (35 mg/kg, i.p) once a week for 8 weeks. Melatonin was given at 5 or 10 mg/kg/day i.p. beginning 4 weeks after the onset of DEN administration and ending at the sacrifice (at 10, 20, 30 or 40 weeks). We investigated whether melatonin has antiproliferative effect through the modulation of the nuclear receptor subfamily 1 group D (REV-ERB), a repressor of BMAL1, by using a REV-ERB agonist, SR9009, in human Hep3B cells. Melatonin cell groups (0.5 or 1 mM) were exposed or not to SR9009 (10 μ M) for 24 hours. To further

assess the contribution of melatonin on the circadian clocks, BMAL1 was knocked down with siRNA in Hep3B cells that were treated with or without melatonin (0.5 or 1 mM) for 24 hours after transfection. Western blot, qRT-PCR, immunohistochemistry, and cell proliferation assays were performed. Carcinogen administration led to increased mRNA and protein expression of CLOCK, BMAL1, and the retinoic acid receptor-related orphan receptor alpha (ROR α), a positive regulator of BMAL1, from the first time-points of the experiment. BMAL1 immunohistochemistry confirmed a higher expression when DEN was administered. Melatonin significantly reduced these dysregulated levels. By contrast, REV-ERB α and β , CRY1, PER1, PER2, and PER3 decreased following DEN administration reaching the smallest expression in the last study periods. Interestingly, melatonin prevented this disruption, also supported by REV-ERB α immunohistochemistry. The suppression of proliferation induced by SR9009 was potentiated by melatonin in Hep3B cells. Furthermore, BMAL1 knockdown decreased cell proliferation, and increased the expression of the apoptotic markers Bcl-2-associated X protein (Bax), cleaved caspase 3, and poly(ADP-ribose) polymerase 1 and 2 (PARP1/2). However, melatonin effects were attenuated in BMAL1-depleted cells. Results support a contribution of circadian clock components to the beneficial effects of melatonin in HCC and highlight the usefulness of strategies modulating the circadian machinery in hepatocarcinogenesis. Supported by AECC and CEPA Fundation.

Circadian clocks, melatonin, hepatocarcinoma, diethylnitrosamine, SR9009

P3-26

MELATONIN ATTENUATES DYSREGULATION OF THE CIRCADIAN CLOCK PATHWAY IN MICE WITH CCL4-INDUCED FIBROSIS AND HUMAN HEPATIC STELLATE CELLS B

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Hepatic fibrosis is a common scarring response to all forms of chronic liver injury, which can result in elevated extracellular matrix protein production by activated hepatic stellate cells (HSCs). The liver expresses a diverse set of genes in a circadian manner and may be under direct or indirect circadian control. Increasing evidence involucre circadian clock alterations in the fibrogenic process. The main regulator of clock genes is the heterodimer BMAL1-CLOCK, which regulates the expression of transcriptional repressors cryptochrome (CRY) and period (PER). The nuclear receptor REV-ERB plays pivotal role in the modulation of the circadian rhythm as a negative regulator. Melatonin is a secretory product of the pineal gland that, in addition to regulating circadian rhythms, exhibits antifibrotic features. The aim of this study was to determinate the effect of melatonin on the circadian clock pathway disturbances in liver fibrosis. Fibrosis was induced in C57BL/6J mice with CCl4 twice a week for 4 or 6 weeks. Melatonin was given at 5 or 10 mg/kg/day i.p. beginning 2 week after the start of CCl4 administration. We investigated whether melatonin has its antifibrotic effect through the modulation of the REV-ERB α expression, using a REV-ERB agonist, SR9009, in human HSCs line, LX2. Cells were exposed to the ligand at 10 μ M during 24 h. Melatonin groups received the indole at 100 or 500 μ M two hours after SR9009 treatment. To further assess the contribution of melatonin on the circadian clocks, REV-ERB α was knocked down with siRNA in LX2 cells that were treated with or without melatonin (100 or 500 μ M) for 24 hours after transfection. The expression of circadian clock and fibrotic

markers was analyzed by qRT-PCR, Western-blot and immunohistochemistry. The expression of CLOCK, BMAL1, REV-ERB, PERs and CRY's genes were deregulated in CCl4 animals, reaching the highest level at 6 weeks. These alterations were significantly prevented in animals receiving melatonin, at both doses. REV-ERB α expression was significantly increased in CCl4 animals, and melatonin abolished this effect. SR9009-treated LX2 cells showed an increase in the expression of clock markers, which were similar to those observed in cells receiving melatonin. The high expression of fibrotic makers in LX2, were abrogated in SR9009-treated cells and also, in LX2 after REV-ERB α knocked down. In summary, results indicate that melatonin ameliorates the dysregulation of clock genes, which may contribute to the attenuation of liver fibrosis in mice and human hepatic stellate cells. Supported by AECC and CEPA.

Melatonin, clock, liver fibrosis, stellate cells

P3-27

ROLE OF TRANSPORTOME IN CHEMORESISTANCE OF HEPATOBLASTOMA

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Introduction and Aim: Hepatoblastoma (HB) is the predominant form of pediatric liver cancer. Although 80% of HB patients respond to standard neoadjuvant preoperative chemotherapy based on doxorubicin and cisplatin, approximately 20% of the cases have very poor response and prognosis. The lack of response can be due to the presence of mechanisms of chemoresistance (MOCs), including the reduction of intracellular content of drugs resulting from changes in uptake/efflux proteins involved in the transport of drugs. The aim of this study was to determine the role in HB chemoresistance of the so-called “transportome”, i.e., the set of plasma membrane transporters expressed at a given time by tumor cells. **Material and Methods:** Genes involved in drug transport were analyzed by RNA-sequencing in 18 HB specimens and their matched non-tumor (NT) tissues. Selected genes were further studied by RT-QPCR, western blot and immunofluorescence in HB-derived HepG2 and HuH6 cell lines in basal conditions and after 72 h of exposure to cisplatin and doxorubicin. Drug sensitivity in HB cell lines was determined by Sulforhodamine B test. Transport activity of ABC efflux pumps was analyzed by flow cytometry using specific substrates and inhibitors.

Results: Significant changes in the expression of drug transporters were found in HB as compared with surrounding NT tissue. SLC01B1/3 (OATP1B1/3) and SLC22A1 (OCT1) genes were markedly down-regulated in tumors, and lower levels correlated with worse prognosis. Regarding ABC pumps, several ABCC transporters were highly expressed in HB. Interestingly, ABCC4 (MRP4) was up-regulated in tumors of patients with more aggressive phenotype (C2 vs C1). The expression levels of uptake (SLCO1B1, SLC01B3, SLC22A1, SLC22A3, SLC31A1) and efflux (ABCB1, ABCC1-5, ABCG2) transporters in cell lines suggested that both HuH6 and HepG2 cells presented a multidrug resistant phenotype, since they had very low levels of uptake transporters and high or very high levels of export pumps. Moreover, the

exposure of cells to 0.1 μ M doxorubicin or 5 μ M cisplatin for 72 h increased the resistant-phenotype of HB-derived cells, mainly by up-regulation of ABCG2 (BCRP) in HuH6 and ABCC3 (MRP3) in HepG2 cells.

Conclusion: Drug transportome can play an important role in HB chemoresistance and several transport proteins have been selected as potential candidates to develop novel strategies based on pharmacological therapy to overcome chemoresistance.

Pediatric cancer, drug resistance, drug transporters

P3-28

MITOCHONDRIAL MEDIATED ANTIVIRAL IMMUNITY IN A VIRAL MODEL OF ACUTE LIVER FAILURE

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The Rabbit Hemorrhagic Disease Virus (RHDV) induces a severe disease that fulfils many requirements of an animal model of acute liver failure (ALF). However, a better knowledge of molecular mechanisms contributing to liver damage is required. In this viral model we have been established the importance of autophagy, inflammation, endoplasmic reticulum (ER) stress and apoptosis in the development of the disease. Mitochondria are known to act as central hubs for multiple signal transductions and mitophagy, turnover of mitochondria via selective autophagy pathway, is associated to antiviral innate immune system. Mitochondrial-mediated antiviral immunity depends on the activation of the retinoic acid-inducible gene I (RIG-I) transduction pathway and on the participation of a mitochondrial outer membrane adaptor protein (MAVS). This study investigates the modulation of the innate immune system and mitophagy in an animal model of ALF induced by the rabbit hemorrhagic disease virus (RHDV). Rabbits were experimentally infected with 2 \times 10⁴ hemagglutination units of a RHDV isolate and sacrificed at 18, 24 and 30 hours post-infection (hpi). The expression of genes involved in the development of the innate immune response, TLR3, TLR4, RIG-I, TRIF, IFN γ , Granzyma, Perforin, MAVS and NKG2D, was determined. In addition, to determine the role of mitophagy in the ALF model, the expression of PINK, FUNDC and BNIP3 was determined, as well as the presence of autophagic vesicles with mitochondria by transmission electron microscopy (TEM). In rabbits infected with RHDV there were a significant increase in the expression of innate immunity-related genes from 18 hpi compared with non-infected animals. The increase in the expression of mitophagy genes started at 18 hpi and maximum expression was reached at 30 hpi associated with an significant enhance of vesicles with mitochondrias observed by TEM. Findings from the present study could contribute to understand how the physiologic functions of mitochondria are coupled with its functions in antiviral immunity, and to clarify the link between mitochondrial mediated immunity and liver damage.

Mitophagy, immunity, acute liver failure

P3-29

INHIBITION OF ABC EXPORT PUMPS BY SESQUITERPENES INCREASES LIVER CANCER SENSITIVITY TO CHEMOTHERAPY

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Liver cancer constitutes a growing challenge with increasing morbidity and mortality, which is partly due to its marked resistance to chemotherapy. ATP-binding cassette (ABC) transporters play a key role in multidrug resistance (MDR) phenotype, both in hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). Therefore, inhibiting these pumps may be useful for restoring the sensitivity of resistant tumors to chemotherapy. Many natural compounds have been found to behave as chemosensitizers *in vitro* by inhibiting ABC-transporters. Here, we have evaluated both *in vitro* and *in vivo* the usefulness of some natural sesquiterpenes to improve the response of HCC and CCA to sorafenib. A human hepatoma PLC/PRF/5/R chemoresistant subline, which expresses high levels of ABC proteins (MDR1, MRP1, MRP2, MRP4 and MRP5), and human cholangiocarcinoma TFK-1 and EGI-1 cell lines, which highly express MRP3 and BCRP, were used to test five natural sesquiterpenes that were selected based on their chemical structure and pharmacological properties. The effect of β -caryophyllene oxide (CRYO), β -caryophyllene, α -humulene, nerolidol and valencene on ABC-mediated efflux of pre-loaded fluorescent substrates (rhodamine for MDR1, calcein for MRP1 and MRP2, fluorescein for MRP3, MRP4 and MRP5 and mitoxantrone for BCRP) was determined by flow cytometry after 30 min. The strongest inhibition was obtained by incubating the cells with CRYO both before and during the efflux period. In PLC/PRF/5/R cells, CRYO inhibited MDR1 and MRP1/2 but not MRP3/4/5. In TFK-1 and EGI-1 cells, CRYO inhibited BCRP, resulting more effective when used in pre-treatment protocol. We also evaluated the usefulness of non-toxic concentrations of CRYO to inhibit ABC pumps and improve the response of HCC cells to sorafenib in both wild-type and chemoresistant sublines of PLC/PRF/5, mouse hepatoma Hepa 1-6 and human lung carcinoma COR-L23 cells. In all cases, CRYO inhibited sorafenib efflux, increased its intracellular accumulation (HPLC-MS/MS analysis) and potentiated its cytotoxic effect (MTT assay). The chemosensitizing effect of CRYO in combination with sorafenib was investigated in nude (nu/nu) mice after subcutaneous injection of Hepa 1-6 cells. The treatment with sorafenib (10 mg/kg, *i.p.*, 2 days a week for 21 days) moderately inhibited tumor growth, while its combination with CRYO (50 mg/kg) produced a stronger tumor inhibition. In conclusion, the combined treatment of CRYO with sorafenib is a useful strategy to improve the effectiveness of low-dose sorafenib chemotherapy and hence reduce its adverse effects. These results support the potential pharmacological interest of CRYO as a new chemosensitizing agent to overcome MDR phenotype in liver cancer.

Multidrug resistance, ABC- proteins, chemosensitizer, sesquiterpenes

P3-30

ROLE OF HYPOXIA IN A HEPATOCELLULAR CARCINOMA IN VITRO MODEL OF ACQUIRED RESISTANCE TO SORAFENIB

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Background and aim: sorafenib is the only drug approved as first-line treatment for advanced hepatocellular carcinoma (HCC). However, its efficacy is limited due to the development of resistant tumor cells. Hypoxic microenvironment is one of the main mechanisms responsible for acquired resistance to sorafenib. The most common response adopted by resistant cells under hypoxia is the activation of the hypoxia-inducible factors 1 and 2 alpha (HIF-1 α and HIF-2 α), which regulate multiple genes involved in tumor survival. Our aim was to characterize the implication of sorafenib resistance in the growth rate, hypoxia response and apoptotic cell death in HCC cells.

Methods: to study sorafenib resistance we used the human HCC cell line HepG2 and two variants of this cell line which undergo resistance to sorafenib (HepG2S1 and HepG2S3, kindly provided by Prof. Van Pelt, Belgium). Resistant cells were continuously cultured in the presence of 6 μ M sorafenib to preserve drug resistance and hypoxia was induced with the hypoximimetic CoCl₂ (100 μ M). Growth dynamic was assessed using crystal violet staining; hypoxia and apoptosis proteins levels were measured by Western blot; cell cycle distribution was analyzed by flow cytometry (FACS); and GraphPad Prism 6 software was employed to perform the statistical analysis, considering significant differences when $p < 0.05$.

Results: sorafenib resistant cell lines HepG2S1 and HepG2S3 showed a more aggressive growth than HepG2 parental cells under normoxia and hypoxia conditions, while sorafenib treatment avoided HepG2 proliferation. Nevertheless, stress by hypoxia decreased growth in all cells analyzed, being this reduction higher in parental cells, which suggests that HepG2S1 and HepG2S3 could have adaptive mechanisms against hypoxia. These results are consistent with HIF-1 α and HIF-2 α overexpression in resistant cells, which was even detected under normoxia. Sorafenib increased the percentage of HepG2 cells in G2-phase that is related to apoptosis induction through Bax and cleaved-caspase 3 protein levels increase. However, HepG2S1 and HepG2S3 cell lines presented a different cell cycle distribution in which the number of G1-phase cells was markedly increased in detriment of G2-phase, resulting in apoptosis arrest.

Conclusion: sorafenib resistance confers a more aggressive phenotype to HCC cells probably because of a robust activation of hypoxia response mechanisms even under normoxic conditions, inducing cellular growth and evading apoptosis. The overexpression of HIFs under normoxia could reflect an important prosurvival mechanism developed by sorafenib resistant cells.

Hepatocarcinoma, HIF, hypoxia, resistance, sorafenib

P3-31

IMPAIRED EXPRESSION IN LIVER AND PLACENTA OF KEY GENES INVOLVED IN PROGESTERONE METABOLISM DURING CHOLESTASIS OF PREGNANCY

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is characterized by elevated bile acid concentrations in maternal serum. This is accompanied by phenotypic changes associated with an abnormal

metabolism of sex hormones. Thus, progesterone and some of its metabolites have been found elevated in serum of women with ICP suggesting a role in the multifactorial aetiopathogenesis of ICP. Enzymes belonging to hydroxysteroid dehydrogenase (HSD) family, such as aldo-keto reductases (AKRs) and short-chain dehydrogenases (SDRs) are involved in the production of these metabolites catalyzing key biotransformations of active steroidal hormones into their inactive derivatives.

Aim: To investigate the effect of maternal cholestasis on placental and hepatic expression of key genes involved in progesterone metabolism.

Methods: As an experimental model of hypercholanemia during pregnancy in rats, complete obstructive cholestasis from day 14 of gestation to term (day 21) was used. Placenta and liver samples were collected at term. The mRNA and protein levels of main enzymes involved in metabolism of sex hormones were determined by RT-qPCR and western blot, respectively. The catalytic activity of Akr1c2 in liver homogenates was determined by measuring the rate of 5 α -dihydroprogesterone disappearance as analyzed by HPLC-MS/MS.

Results: In maternal liver, cholestasis did not affect the expression levels of proteins involved in the metabolism of progesterone, except for Srd5a1 and Akr1c2, that convert progesterone into its inactive metabolites 5 α -DHP and allopregnanolone (PM4), respectively, which were down-regulated. This was accompanied by a similar decrease (~50%) in protein levels and enzymatic activity of Akr1c2. In contrast, in placenta, Srd5a1, Akr1c2 and other enzymes involved in progesterone catabolism such as Cyp17a1 and Cyp21a1, were up-regulated during maternal cholestasis. 3 β -Hydroxysteroid dehydrogenase, involved in the last step of progesterone biosynthesis, whose expression showed a trend to increase in liver, was significantly reduced in placenta. To elucidate whether changes found in Akr1c2 and Srd5a1 might be under Fxr control, their expression was determined in the liver of cholestatic Fxr^{-/-} mice. No significant differences between wild-type and Fxr^{-/-} mice were found, suggesting that mechanisms other than Fxr pathway may be involved in the regulation of these enzymes during cholestasis.

Conclusion: Through an Fxr-independent mechanism, ICP induces an up-regulation of enzymes involved in the inactivation pathways of progesterone in the placenta, together with a decrease in their hepatic expression and catalytic activity. These changes might act as an adaptive mechanism to counterbalance the accumulation of progesterone, thus protecting the fetus from high concentrations of this hormone during ICP.

Cholestasis of Pregnancy, Progesterone, Placenta

P3-32

THE MELATONIN-RECEPTOR ANTAGONIST LUZINDOLE INDUCES CA²⁺ MOBILIZATION AND REACTIVE OXYGEN SPECIES GENERATION IN MOUSE PANCREATIC ACINAR CELLS

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Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine produced in many organs within the body. The indole is produced following circadian rhythm with high levels being released at night and has been implicated in the regulation of physiological processes in major tissues,

including the pancreas. Melatonin MT1 and MT2 receptors are G-protein-coupled receptors, which are expressed in the gastrointestinal tract, in addition to other tissues and organs. Luzindole (N-acetyl-2-benzyltryptamine), is a selective melatonin receptor antagonist, and has been widely employed to unravel the signaling pathways and the associated neuroendocrine and functional responses induced by melatonin in mammals. It has been described as an antagonist of melatonin MT1 and MT2 receptors. In this study we have examined the effects of luzindole (1 μ M, 5 μ M, 10 μ M and 50 μ M) on isolated mouse pancreatic acinar cells. Cells were prepared by collagenase digestion of samples of mouse pancreas. Fluorimetric analysis of fura-2-loaded cells was used to follow changes in intracellular free Ca²⁺ concentration ([Ca²⁺]_i). Production of reactive oxygen species (ROS) was monitored following CM-H2DCFDA and MitoSOXTM Red-derived fluorescence. Stimulation of mouse pancreatic cells with the secretagogue cholecystokinin (1 nM) evoked a transient increase in [Ca²⁺]_i. In the presence of luzindole increases in [Ca²⁺]_i were observed, which consisted of either a slow increase towards a plateau or of single, short-lasting, increases of Ca²⁺ concentration. Treatment of cells with the sarco-endoplasmic reticulum Ca²⁺-ATPase inhibitor thapsigargin (1 μ M), in the absence of Ca²⁺ in the extracellular medium, evoked a transient increase in [Ca²⁺]_i. The additional incubation of cells with luzindole (10 μ M) failed to induce further mobilization of Ca²⁺. In the presence of luzindole a concentration-dependent increase in ROS generation was noted, both in the cytosol and in the mitochondria. Finally, pretreatment of cells with melatonin (100 μ M) diminished ROS generation evoked by luzindole. In conclusion, our findings show that inhibition of melatonin-receptors induces Ca²⁺ mobilization from intracellular stores. In addition, probable pro-oxidative conditions are observed. Altogether our results suggest that inhibition of melatonin receptors creates a situation in pancreatic acinar cells that might compromise their function.

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Luzindole, melatonin, calcium, ROS, exocrine pancreas

P3-33

EFFECT OF ELONGATION FACTOR 2 (eEF2) DEPLETION ON THE GROWTH OF HUMAN ESOPHAGEAL CANCER CELLS AND THE SENSITIVITY TO DOXORUBICIN IN VITRO

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Introducción: Cancer is one of the leading cause of death in worldwide and a major public health problem. Multiple squamous cell carcinomas frequently arise in the esophagus and the head and neck region. Esophageal cancers, including esophageal squamous cell carcinomas more prevalent in Asia and esophageal adenocarcinoma more prevalent in United States and Europe, is the 5th highest causes of cancer mortality worldwide. Esophageal carcinoma is a highly lethal malignancy with a poor prognosis and low survival rate. Upregulation of Elongation factor 2 (eEF2), cytoplasmic component of the protein synthesis machinery that catalyzes the movement of the ribosome along the mRNA, has been reported in different human cancer. Doxorubicin is a chemotherapeutic drug widely used for the treatment of advanced esophageal squamous cell carcinomas. However, cancer patients who receive Doxorubicin treatment can expect chemoresistance. **Objective:** Study the potential role of eEF2 as a marker for esophageal cancers as well as the molecular mechanisms responsible of upregulation of eEF2 in carcinogenesis.

Methods: To determinate the role of eEF2 depletion on proliferation of human esophageal squamous carcinomas cell line and the sensitivity to Doxorubicin *in vitro*, we performed a knockdown of eEF2 by lentivirus-shRNA and cell proliferation and the capacity to form colonies were examined.

Results: Our results showed that depletion of eEF2 result in decrease of cell proliferation and the capacity to form colonies. Moreover, we also examined the possible role of eEF2 and Doxorubicin-induced cell death. We found that eEF2 depletion decrease Doxorubicin chemosensitivity, because a reduction of both cell proliferation and colony formation was found in eEF2 depleted cells.

Conclusion: These findings suggest that eEF2 could be a potential marker and pharmacological target of esophageal cancers and chemoresistance.

Elongation factor 2, Doxorubicin, cancer

P3-34

MODULATORY EFFECTS OF RIFAXIMIN ON INNATE IMMUNE PARAMETERS ON A MURINE MODEL OF DEXTRAN SULFATE SODIUM (DSS)-INDUCED COLITIS

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Background & Objectives: Rifaximin is a wide-spectrum antibiotic that ameliorates intestinal inflammation in Inflammatory Bowel Disease (IBD). Although a modulatory action on local immune responses has been suggested, the mechanism of action of Rifaximin mediating its anti-inflammatory effects remains unclear. Here, we characterized effects of Rifaximin in innate immune parameters related to host:bacterial interactions [namely Toll-like receptors (TLRs) and antimicrobial peptides] in a murine model of colitis.

Methods: Dextran sulfate sodium (DSS, 3%, added to the drinking water during 5 days followed by a 2-day recovery period) was used to induce colitis in adult C57BL/6 female mice. Throughout the same period, animals were treated, in a preventive manner, with Rifaximin (50 or 150 mg/kg/day, PO) or Doxycyclin (30 mg/kg/day, PO). Clinical signs were assessed daily. At necropsy, signs of colonic inflammation and local expression (qRT-PCR) of TLRs (2, 3, 4, 5 and 7), antimicrobial peptides (DEF α 24, RELM β and RegIII γ), inflammatory markers (IL-6, IFN γ , TNF α , IL-1 β , RANTES and IL-10) and pregnane X receptors (PXR) were assessed.

Results: Animals receiving DSS showed clinical signs indicative of the development of colitis. Regardless the dose tested, Rifaximin did not affect the clinical course of colitis, while Doxycyclin, used as a positive control, attenuated clinical signs. Similarly, colitis-associated up-regulation of inflammatory markers was attenuated by Doxycyclin but not modified by Rifaximin. Only TLR5 expression was altered during colitis, showing a minor down-regulation. Treatment with Rifaximin in colitic mice resulted in a down-regulation of TLR3, 4 and 5 and an up-regulation of TLR7. Colitis up-regulated RegIII γ (>100-fold up-regulation), while other antimicrobial peptides assessed were unaffected. Rifaximin did not affect colitis-associated RegIII γ up-regulation, while Doxycyclin completely normalized its expression levels. Expression of PXR was down-regulated during colitis, a change not affected by Rifaximin but prevented by Doxycyclin. Rifaximin, by itself, had a tendency to down-regulate PXR expression.

Conclusions: Results obtained show that Rifaximin is devoid of anti-inflammatory effects in the murine model of DSS-induced colitis. Nevertheless, changes in TLRs expression suggest that Rifaximin

might modulate host:bacterial interaction systems during colitis; although this effect is not associated to an amelioration of inflammation.

Rifaximin, Intestinal Inflammation, Colitis, TLR

P3-35

THE ROLE OF CELLULAR SENEESCENCE IN BISPHENOL A-INDUCED ACUTE KIDNEY INJURY IN WISTAR RATS

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Bisphenol A (BPA) is one of the main volume chemicals produced worldwide. Extensive literature has raised many concerns about its possible implications in the origin of some chronic diseases such as kidney diseases. Physiologically established pharmacokinetic models suggest that renal tubular reabsorption of BPA conjugates contribute to serum BPA levels, but the contribution of this pathway to renal injury hasn't been deeply studied. Proximal tubule cells (PTCs) in renal cortex, which reabsorb many filtered molecules, are the primary site of kidney injury associated with nephrotoxicity. In cellular senescence, interactions of lipofuscin with cellular functions are able to increase its rate of formation, resulting in a vicious cycle, causing cellular malfunction and death. IGF-1 plays a protective role from apoptosis and regulation of cell growth, which facilitates recovery from acute kidney injury. The goal of the present work was to evaluate the effect of short-term treatment with low doses of BPA on cellular senescence in the adult Wistar rat kidney. This study was performed in accordance with guidelines established by Institutional Animal Care and Use Committees at the University of Oviedo (PROE-15/2016). Male Wistar rats (aged 4 months) were subcutaneously injected with vehicle (tocopherol-stripped corn oil, CONTROL group), 50 or 500 μ g/kg/day BPA for a week (BPA50 and BPA500 groups, respectively). The kidneys were fixed in 4% buffered formaldehyde, processed for paraffin embedding and stained, followed by semi-quantitative histological and immunohistochemical analysis with immunoreactive score (IRS) in several microscopic fields (Olympus BX63 microscope, CellSens image analysis software). The visualization signal of anti-IGF-1 was developed with diaminobenzidine in 5 μ m sections. We visualize green lipofuscin's auto-fluorescence emissions at 450-490 nm in 1 μ m optical section by Leica confocal microscope. We detected lipofuscin auto-fluorescence deposits in PTCs as a possible biomarker to detect stress-induced premature senescence in kidneys. Renal cortex shows diffuse and cytosolic lipofuscin's auto-fluorescence in PTCs of BPA50 (1+, weak intensity) and BPA500 treated males (3+, strong intensity), while CONTROL males did not show signal. Cytosolic labelling in cortical PTCs with anti-IGF-1 antibody shows 0 IRS in CONTROL males and 4 and 6 IRS in BPA50 and BPA500 treated males, respectively. Further studies are need to clarify the potential role of BPA in the pathogenesis as well as in the progression of renal diseases.

Kidney, bisphenol A, Wistar Rat

3-36

DIFFERENT EXPRESSION PATTERN OF AQUAPORIN-1 AND AQUAPORIN-3 IN MELANOCYTIC AND NON-MELANOCYTIC SKIN TUMORS

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Aquaporins (AQPs) are a family of small integral membrane proteins that allow water and other small molecules to cross cell membranes. There are at least 13 homologous members in human cells, being AQP3 the most abundant AQP in human epidermis, specifically in keratinocytes. AQP1 have been detected in melanocytes fibroblasts and vessels of skin. Recent works pointed toward an important role of AQP in angiogenesis, cell proliferation and cell migration, all necessary process for tumorigenesis. We studied here the expression of AQP1 and AQP3 by immunohistochemical staining of 72 skin biopsies obtained from patients diagnosed for a primary melanocytic skin tumor (melanocytic naevus, atypical melanocytic naevus, or melanoma), skin nonmelanocytic tumors (Basal cell carcinoma or Squamous cell carcinoma) or healthy control samples. Staining for AQP1 showed strong labeling in 100% (7/7) melanocytic nevi. The small blood vessels, stroma and melanophages surrounding different types of melanomas tumors also showed positive signal. However the tumoral melanocytes in atypical nevi (5/5) and melanomas (20/20) were consistently negative for AQP1. On the other hand and despite AQP3 have not been described in melanocytes, staining for AQP3 showed strong labeling in 100% (7/7) melanocytic nevi, 100% (5/5) atypical melanocytic nevi, 100% of melanomas: (5/5) lentigo maligna melanoma, (6/6) superficial spreading melanoma, (5/5) nodular melanoma and (4/4) acral lentiginous melanoma. In all basal cell carcinoma (BCC) and Squamous cell carcinoma (SCC) staining for AQP1 was negative, meanwhile AQP3 resulted positive in all cases. This work corresponds with the first demonstration of AQP1 and AQP3 expression in human specimens of melanocytic skin tumors. More studies are needed to understand the underlying molecular mechanisms of expression of AQP1 and AQP3 in melanocytic tumors and their possible potential as molecular targets in the treatment of these neoplasms.

Aquaporins, skin cancer, naevus, melanoma

P3-37

ENERGY METABOLISM IN HUMAN PROXIMAL TUBULE CELLS UNDER VARYING GLUCOSE CONCENTRATIONS

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Renal damage, either acute or chronic, is one of the leading causes of mortality and morbidity in our society. End-stage renal disease is a prevalent condition with a high social and economic burden. In vitro models of renal proximal tubule, the most damage-sensitive segment in the nephron, are in demand. In this report we summarize our study of energy metabolism in a human proximal tubule cell line. RPTEC-TERT1 (RPTEC), trans-formed human renal cells immortalized by expressing the human telomerase, maintain phenotypic features of differentiated proximal tubule. However, we have found RPTEC express markers of other nephron segments or even markers of epithelial-to-mesenchymal transition. RPTEC providers recommend growing RPTEC in DMEM-F12 mixture which

contains 17 mM glucose (300 mg/dL), which is >3-fold the physiological concentration of glucose. Thus, we studied the phenotype and energy metabolism of RPTEC cells grown in varying glucose concentrations, with the goal to optimize physiological culture conditions. We found that RPTEC grown in 5 mM glucose proliferate at the same rate (doubling time 2.5-3 days) and express a similar transcriptomic profile as those grown in 17 mM. Small differences were found in the use of glucose (5.2 ± 0.8 vs 3 ± 0.9 pmol/h/cm²) and production of lactate (2.1 ± 0.2 vs 2 ± 0.9 pmol/h/cm²). Oxygen consumption rates (OCR) and mitochondrial/nuclear DNA ratios were similar. We thus conclude RPTEC cells can be safely cultured in 5 mM glucose. Because RPTEC cells exhibit glycolytic metabolism, unlike proximal tubule cells in vivo, we tested whether RPTEC would survive and maintain or improve their differentiated state if grown in a glucose-free medium (with pyruvate and glutamine as main fuels). RPTEC could only be transferred to 0 mM glucose after confluency had been reached. Thereafter, RPTEC survived in 0 mM glucose for at least ten days, although cell number was significantly lower than that observed in 5mM (0.16 ± 0.02 vs 0.34 ± 0.08 cells/cm²), and the presence of domes (a marker of transepithelial transport) was abrogated. OCR (6.6 ± 3 vs 5.2 ± 1 fmol/min/106 cells) and mt/nuclear DNA (3896 ± 880 vs 2713 ± 1156 copy number ratio) was increased in cells maintained in 0 mM glucose, which, together with a low lactate production (0.34 ± 0.5 pmol/h/cm²) suggested a predominant oxidative metabolism. Our preliminary data showed no differences in transcriptomic profile. A deeper analysis will establish under which glucose concentration RPTEC cells best mimic proximal tubule physiology and pathology. Funded by Spain; Ministerio de Economía, Industria y Competitividad DPI2015_65401.

Proximal tubule, renal damage, cell metabolism

P3-38

BINGE DRINKING AFFECTS HYDRIC BALANCE AND ALDOSTERONE LEVELS INCREASING SYSTOLIC BLOOD PRESSURE IN ADOLESCENT RATS

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Introduction: Binge drinking (BD) consumption during adolescence has been associated with higher risk of hypertension and cardiac arrhythmia. The mechanism by which BD provokes HTA is not well elucidated; however it seems that one of them is the stimulation of Renin-Angiotensin-Aldosterone System (RAAS).

Objectives: Evaluate, in adolescent rats, aldosterone levels and its clearance, Na⁺ and K⁺ reabsorption as well as systolic blood pressure (SBP). **Methods:** Teenagers rats used in this study were divided in control and BD groups. Alcohol solution (20% v/v) was administered intraperitoneally (3 g/Kg/day during 3 days/week for 3 weeks) to BD group. Control group received an intraperitoneal injection of an equal volume of isotonic saline solution during the same time. Aldosterone, Na⁺, K⁺ and creatinine levels in serum and urine were determined. Aldosterone and creatinine clearances, fractional excretion of sodium (FENa⁺) and transtubular potassium gradient (TTKG) were calculated. SBP was determined by tail occlusion method.

Results: BD increased Na⁺ and decreased K⁺ serum concentrations. Also, BD decreased creatinine clearance and FENa and increased TTKG and aldosterone serum levels; it also drastically reduces relative aldosterone clearance and increased SBP.

Conclusion: BD leads to a lower glomerular filtration rate since creatinine clearance is reduced and causes hypernatremia, hypokalemia and hyperaldosteronemia along with a high Na⁺ renal reabsorption and a high

K⁺ urine secretion, and even to a reduction in aldosterone urine excretion related to a lower relative aldosterone clearance value. In summary BD exposition in adolescent rats triggers hydric and electrolytes imbalances which contribute to increase SBP.

Binge drinking, adolescent, aldosterone, hypertension

P3-39

OBESITY AND SPERM CONCENTRATION OF METALS: ASSOCIATION WITH SEMEN QUALITY

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Introduction: A decrease in male fertility has been reported. Some studies estimate that sperm counts continue to decrease by up to 1.5% per year. Because this decline in fertility is produced in parallel with the rising rates of obesity, this pathology has been postulated as one of the causes of male infertility and reduced fertility. Also, other environmental factors such as metals present in water, soil, dust and diet have an impact on sperm parameters. **Objective:** To study the effect of obesity and the concentration of metals in spermatozoa on the seminal quality. **Method:** Seminal sample from normozoospermic controls (Cn; n = 17; BMI: 24.6 ± 2.0) and obese subjects (Ob; n = 17; BMI: 32.6 ± 4.4) were analyzed. Seventeen metals (Mg, Sc, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Y, Au and Pb) were measured by ICP-MS. Differences in semen quality were also evaluated. The results were analyzed with Mann–Whitney test and the association between variables was performed with Rho Spearman test.

Results: In relation to sperm quality parameters, significant statistical differences were found in the analysis of progressive sperm motility (p=0.007), immotile sperm (p=0.02), and tail defects, which accounts for the effects of obesity on motility spermatic (p=0.02). Spermatozoa showed an increased concentration of V and Ni in Ob versus Cn (p=0.01 y p=0.006, respectively) and a negative association between both Cr (rho=-0.31; p=0.04) and Ni (rho=-0.32; p=0.03) and the percentage of progressive sperm motility. We observed an association between BMI and Sc (rho=0.32; p=0.03), Cr (rho=0.31; p=0.04), Ni (rho=0.39; p=0.01), Mo (rho=0.36; p=0.02) and Pb (rho=0.36; p=0.02). **Conclusion:** Obesity has an impact on the characteristics of sperm motility and it was correlated with the presence of five metals (Sc, Cr, Ni, Mo and Pb) in spermatozoa. In addition, Cr and Ni were inversely correlated with sperm motility.

Obesity, metals, spermatozoa

P3-40

HAEMOSTASIS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA MURINE MODELS

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Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal-dominant disorder, involving mutations in two predominant genes: Endoglin (ENG; HHT1) and activin receptor-like kinase 1 (ACVRL1/ALK1; HHT2). This disease is characterized by telangiectasias and arteriovenous malformations. Also, patients have frequent and severe bleedings, most common are epistaxis and gastrointestinal bleedings that cause chronic anaemia in approximately one-third of patients and reduces their quality of life. It is admitted that vessel malformations are due to mutation in genes involved in HHT, because they encode proteins that modulate transforming growth factor (TGF)- β and BMP superfamily signalling in vascular endothelial cells, which has a role in angiogenesis. Regarding to bleedings, it is logical to think that malformed vessels imply a greater breakage and, therefore, bleeding. However, this would explain their frequency, not the why of the difficulty in stopping them. Nothing clear enough has been described before about the etiology of this complication. We have studied haemostasis process in two heterozygous mice in both genes, endoglin (Eng+/-) and activin receptor like kinase-1 (ALK1+/-). Eng+/- mice have significative longer bleeding time. A different bleeding profile is observed in ALK1+/- models, they present a higher, but not significant, bleeding time. Thus, HHT1 mice has altered their capacity to stop a haemorrhage. We have analysed every phase of haemostasis to find out what is affected by endoglin deficiency. Nothing seems to be altered, but thrombus stabilization. There are no differences in primary haemostasis, functionality of platelets, neither activation nor aggregation, also fibrinolysis main components have normal levels and it is described that alterations in coagulation cascade factors have never been found. It exists another step, thrombus stabilization, where secondary links help to maintain the new plug stable and endothelial endoglin have a role in it. Our results show that there is significative less adhesion of activated platelets in a monolayer of primary endothelial cells from Eng+/- mice. Also, in these mice, there is a significative delay in the time that is needed to generate a plug stable enough to close blood flow after a local damage. In the other hand, opposite results have been obtained in mice that overexpress endoglin (Eng+). These mice needed less time to stabilize a thrombus big enough to occlude the carotid. Furthermore, bleeding time and platelet functionality are normal. Thus, we confirm that endoglin has a role in haemostasis by interacting with platelet and helping to stabilize new thrombus in the endothelium.

Hereditary hemorrhagic telangiectasia, HHT, hemostasis, endoglin

P3-41

HSP70 INHIBITION SENSITIZES U937 LEUKAEMIA CELLS TO THE COMBINED TREATMENT OF MELATONIN AND ANTICANCER AGENTS

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HSP70 is constitutively expressed to guarantee the correct folding of nascent proteins and prevents the unfolding or misfolding of proteins under stressing conditions. Despite its role is essential for the cell

viability, HSP70 is upregulated when the protein functionality is threatened as part of “stress response”. When the stress lets up, the levels of HSP70 comes down to the normal levels and the cell activity resumes ordinarily. Mild stress or exogenous HSP70 are known to be responsible of increasing HSP levels concomitantly with apoptosis resistance. Lethal stress, which causes molecular alterations that cannot be addressed by the stress response, leads the cell to the apoptotic death. On the other hand, extended stressing environment entails overexpression of HSP70 which is closely linked with the acquirement of stress tolerance, that is to say in some cases the ideal conditions prone to oncogenesis. HSP70 inhibition (pifithrin- μ , P- μ ; 24 h) has been shown as an antineoplastic strategy to treat acute leukaemias, alone or with other anticancer drugs. Previous studies have shown melatonin as an apoptosis enhancer in cancer cells and an apoptosis inhibitor in non-tumour cells. Additionally, melatonin has been combined with chemotherapy agent reaching a synergistic response. The goal, herein, is to study the role of HSP70 in the resistance to combined treatments of anti-cancer drugs (5-fluorouracil, etoposide, cytarabine) and melatonin in the leukaemia U937 cell line. To this aim U937 were pre-treated 24 h HSP70 inhibitor (P- μ) and/or 1 mM melatonin. U937 cells did not display changes in their proliferation (Neubauer cell counting) under HSP70 inhibition, however 1 mM melatonin (24 h) entailed a diminished cell counting. The combination P- μ and melatonin showed additive inhibition of cell proliferation. According to our previous results, MTT assay of cell viability indicates that melatonin played an enhancer role in anti-cancer drug-induced apoptosis, as corroborated by nuclear morphology (Hoescht 33342). Nonetheless, such effect was still strengthened when HSP70 was inhibited. HSP70 inhibition might represent the Achilles’ heel of the apoptosis resistance to approach more efficient treatments. Given that it is widely known the protection capacity of melatonin in non-tumour cells under toxic agents, the inhibition of HSP70 might sensitize leukaemia cells to the combination of melatonin and anti-cancer agents, and likely reducing the side effects.

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Apoptosis resistance, HSP70, melatonin, combined chemotherapy

P3-42

BLOOD PLASMA POLYPHOSPHATE LEVELS ARE LOWER ON HIGH THROMBOTIC RISK PATIENTS THAT HAVE THE FACTOR V LEIDEN MUTATION

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Polyphosphates are natural moieties that are accumulated in high concentration in human platelets. They have been recently recognized as potent prothrombotic, procoagulant and proinflammatory agents. Functional studies with polyphosphate have been done mostly using in vitro and animal experimental models. Previous research suggested that plasma polyphosphate may be elevated in high thrombosis pathologies, however, levels of this polymer were unknown because there was not an accurate assay to determinate them. Among the inherited prothrombotic factors, the Factor V Leiden mutation is the most frequent in Caucasian populations. Factor V is an essential promoter of the last step on the coagulation cascade. As mutated Factor V is resistant to be degraded (by activated

protein C), this abnormality results in a significative increment of thrombosis. Carriers of Factor V Leiden mutation have 5-80-fold increased risk of venous thromboembolism. Recently, our group developed an effective procedure to determinate blood plasma polyphosphates, using the plasma ‘crioprecipitated’ protein fraction. This procedure concentrates nearly all polyphosphates and eliminates the contamination with phosphate in the samples (Santi et al., 2016 *Thromb Res.* 144:53-5). Then, using this new method we report here the study of 40 patients that have the Leiden mutation in coagulation Factor V. In our study, diagnostic of Leiden mutation in Factor V was confirmed by genetic analysis using PCR and fragment analysis. All 40 patients were heterozygous carriers. Paradoxically, median levels of blood plasma polyphosphate were 40 times lower in patients with the mutation compared with healthy participants. The decrement of prothrombotic polyphosphate in high-risk thrombosis patients suggests the presence of compensatory mechanisms to maintain homeostasis. We are now investigating the clinical records of the patients looking for possible associations between polyphosphate levels and other biochemical parameters and treatments.

Blood, polyphosphates, Factor V

P3-43

MELATONIN SENSITIZES U937 LEUKAEMIA CELLS TO NEW ANTICANCER AGENTS BASED IN PLATINUM AND PALADIUM
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Introduction: Chemotherapy is one of the therapeutic methods more used for cancer treatment; the goal is to inhibit the tumour growth and/or destroy cancer cells. The efficiency of chemotherapeutic agents depends, in many cases, on the type of chemotherapeutic uses and on the target tissue. The development and the synthesis of new chemotherapeutic agents with organic ligands or different metallic ions can make them active against some types of cells and not against others. On the other hand, many studies have indicated that melatonin is an antiproliferative, oncostatic, antioxidant, antiaging and immunoregulator agent, besides, it has antiapoptotic effects in non tumour-cells, as proapoptotic effects in some types of tumour cells, although the molecular mechanism involved in these effects is still unknown.

Objective: this research aims to study the anti-leukaemia effects of the combined treatment of melatonin with new generation of chemotherapeutic agents.

Materials and method: U937 cells were used as a model of Acute Myeloid Leukemia (AML). 1 mM Melatonin was administered to U937 cells 24 hours before the treatment with these new chemotherapeutics. Then cells were treated for 4 h with Chemotherapeutic agents TdTn (2-(3,4-diclorofenil)imin-N-(2-tiazolin-2-il)tiazolidin) ligand, ligand joined with a molecule of platinum (PtTdTn) (dichlorine-(2-(3,4-diclorofenil)imino-N-(2-tiazolin-2-il)tiazolidin)-platino(II)) or joined to one of palladium (PdTdTn) (dichlorine-(2-(3,4-diclorofenil)imino-N-(2-tiazolin-2-il)tiazolidina)-paladio(II)) and we used cytarabine as control of anti-leukaemic agent. Viability was analyzed through the MTT technique. Apoptosis was quantified as the percentage of apoptotic nuclei by fluorescence microscopy (Hoescht 33342fluoride). The membrane potential assay was performed with specific probe JC(1).

Results: We found that both melatonin and the new chemotherapeutic agents tested (TdTn, PtTdTn and PdTdTn) induced a decrease in the viability of U937 cells, and a concomitant increase in apoptosis. The combined treatment with melatonin and the anticancer agent induced an additional decrease of cell viability and increase in the percentages of

apoptotic cells. The assay of mitochondrial membrane potential displayed coherent results. In conclusion, melatonin strengthens the anti-leukemic capacity of the new generation therapeutic agents based on Pt and Pd (PtTdTn and PdTdTn).

Conclusion: these results propose the treatment of leukemia with melatonin and the new generation agents based on Pt and Pd as a promising alternative to conventional chemotherapies.

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Leukaemia cells, Melatonin, anticancer agents

P3-44

EFFECT OF ENDOGLIN OVEREXPRESSION ON THE MATURATION AND STABILIZATION OF BLOOD VESSELS: AN HISTOLOGIC POINT OF VIEW

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Angiogenesis is the formation of new capillaries from existing blood vessels that occurs in distinct pathophysiologic processes. Endoglin is a homodimeric membrane glycoprotein that is highly involved in endothelium function and it plays a major role in angiogenesis, as its absence or deficiency produces defects in this process. Previous studies by this research group have showed that continuous overexpression of endoglin (ENG+) produces defects in angiogenesis. Since these alterations do not

affect the initial phases of angiogenesis, we aim to investigate the role of endoglin overexpression on the stabilization and maturation of new blood vessels. In order to create functional vessels, its maturation and stabilization is essential, which involves the recruitment of mural cells and their adhesion to the vessels, as well as, the formation of stable endothelial junctions. To analyze the distribution of mural cells in the new vessels, we used two models of angiogenesis *in vivo*: the retina vasculature of mice pups ENG+ and the *in vivo* Matrigel® angiogenesis assay. In the first model, we performed a co-labeling with lectin, which binds to the endothelium, and NG2, which is a marker of pericytes (mural cell). We found an almost complete colocalization of these two markers in WT retinas, however, in ENG+ retinas the endothelium is not entirely covered by mural cells and pericytes that do not seem to be bound, suggesting an impaired pericyte attachment. In the second model, we detect the new blood vessels in the Matrigel® plugs by immunostaining with anti-CD31 (endothelial cell marker) and anti-NG2 antibodies. This assay confirmed that the vessels from ENG+ plugs have less mural coverage than the vessels from WT plugs. We hypothesized that the lesser maturation of the ENG+ blood vessels could be due to the lack of endothelium stabilization, that depends on the quiescent state of endothelium and the formation of endothelial junctions established by VE-cadherin. To verify this, we analyzed the VE-cadherin distribution in the retina vasculature and observed that the ENG+ retinas have a more irregular distribution and less inactive junctions than the WT. Moreover, we evaluated the proliferation in the central area of the retina where the endothelium is more stable, by immunofluorescence, where we found more proliferating cells in the ENG+ retinas, agreeing with our hypothesis. We can conclude that continuous endoglin overexpression produces unstable vessels with poor mural coverage, which could contribute to increase the vascular permeability and the breakage of the new vessels.

Endoglin, angiogenesis vessel maturation and stabilization

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