



**REUNIÓN ANUAL DE LA
SOCIEDAD ARGENTINA DE
FISIOLOGÍA
SAFIS**

VIII Encuentro de Docentes en Fisiología y Física Biológica

22 al 24 de octubre de 2025

Rosario, Argentina

**ANNUAL MEETING OF THE ARGENTINE
SOCIETY OF PHYSIOLOGY 2025**

Program & Abstracts

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D E H N E R S. R. L.



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Wednesday 22 october			Thursday 23 october			Friday 24 october		
			8:00-18:00	Registration	Hall Plaza Real	8:00-12:00	Registration	Hall Plaza Real
			8:30-10:00	Cardiovascular Symposium "The heart in Focus: Structural Insights, Microbial Influence, and Therapeutic Advances"	Belgrano	8:30-10:00	Camilion de Hurtado Award	Belgrano
			10:00-12:00	Posters Fisiología Cardiovascular - Hipertensión Fisiología Respiratoria Fisiología Gastrointestinal	Belgrano Plaza Real	10:00-11:30	Young investigators Symposium	Belgrano
12:00-13:00	Registration	Hall Fbioyf	12:00-13:00	Lunch time		11:30-12:30	Lunch time	
13:00-13:40	Mini-orals I Curricular Integration Strategies in Physiology Education	Aula 1 Fbioyf	13:00-14:30	Gastroenterology Symposium "Novel Mechanisms in Liver Disease Pathogenesis"	Belgrano	12:30-14:00	Safis Award	Belgrano
13:40-14:20	Mini-orals II ICTs and Teaching Strategies for Physiology Education	Aula 1 Fbioyf	14:30-16:00	Extracellular Vesicles Symposium "Extracellular Vesicles in Physiological and Pathological Conditions"	Belgrano	14:00-15:30	Oncology Symposium "Inside the Cancer Cell: Pathway Disruption, Nuclear Armor, and Sensory Loss."	Belgrano
14:20-14:40	Break	Hall Fbioyf	16:00-16:15	Coffee Break	Plaza Real	15:30-15:45	Coffee Break	Plaza Real
14:40-15:30	Round table The Role of Non-Graduate Teaching Assistants in Physiology Education	Aula 1 Fbioyf	16:00-18:00	Posters Endocrinology, Metabolism and Reproduction Renal Physiology	Plaza Real Belgrano	15:30-17:30	Posters Cell Physiology - Cell Signalling Oncology - Genetics - Gene Therapy - Immunology	Plaza Real Belgrano
15:30-15:45	Coffee Break	Hall Fbioyf	18:00-19:00	Opening Conference "Novel therapies for urea cycle disorders: from understanding metabolic pathways to proof-of-concept studies"	Belgrano	17:30-19:00	Endocrinology Symposium "The Skeletal Muscle: a new endocrine organ?"	Belgrano
15:45-16:25	Mini-orals III Evaluation Practices in Physiology	Aula 1 Fbioyf	19:00-19:30	Former presidents tribute	Plaza Real	19:00-20:00	Closing conference "The role of Metformin in Alzheimer's disease. Experimental evidence and informacton from a global patient database"	Belgrano
16:25-17:05	Reflexive aperture. Rethinking the teaching of Physiology: An integrative proposal	Aula 1 Fbioyf	19:30-20:30	SAFIS General Assembly	Belgrano	20:00-20:30	Awards Ceremony	Plaza Real
17:05-18:45	Workshop "Core Concepts" in Physiology Education	Aula 1 Fbioyf						
18:45-19:00	Informal closing of the Education Meeting	Aula 1 Fbioyf						
19:00-20:30	Scientific Policy Roundtable	Aula 1 Fbioyf						



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D E H N E R S . R . L .



Welcome from the President of SAFIS

Dr. Enrique Sánchez Pozzi

Dear Colleagues,

I extend the warmest welcome to the annual meeting of the Argentine Society of Physiology (SAFIS), which we are holding this year in the city of Rosario.

We are currently facing a critical situation in the scientific and educational fields, marked by the resource limitations imposed by the national government on public science and education. In this context, this congress is not just an event; it is a display of resistance, a clear demonstration that we are alive and active.

And we are alive because the call for papers was remarkable: 67 works were presented in mini-oral format, 17 of them within the valuable Teacher's Meeting (Encuentro Docente). This is the true spirit of resistance exerted by our researchers and fellows, who continue to conduct excellent science despite the scarcity of resources.

Thanks to the subsidy from The Company of Biologists, we were able to organize a hybrid congress. This allowed for active participation from those who could not travel, with [xxx] works presented via *streaming*. Without this format, many of these valuable contributions could not have been discussed.

I profoundly thank all the invited speakers and lecturers for their participation. Their commitment has allowed us to cover a large part of the specialties within Physiology and, above all, to maintain the high scientific level of our meeting.

I want to highlight the value of two fundamental activities of our society: the Teacher's Meeting and the Young Researchers' Symposium. Both are pillars of our identity. The Teacher's Meeting demonstrates our conviction that teaching and research go hand-in-hand. We believe in the necessity of pedagogical updating to continue training future professionals in a constantly and rapidly changing society. My recognition goes to the Education Committee for their efforts in organizing it. Regarding Young Researchers, we encourage them to take an active role in our society and to address their specific issues during the congress. My thanks to the Young Researchers Commission for managing a dedicated space for exchanges and discussion and for ensuring the success of the meeting..

My special thanks extend to the entire Executive Board that accompanies me in the management, particularly Cecilia Basiglio, our secretary, and Daniel Francés, our treasurer. I also acknowledge and appreciate the indispensable administrative support of our secretary, Valeria Casazza, for carrying out the daily management of the society.

Finally, it is crucial to highlight the support of the institutions and entities that made this event possible.

We especially thank: CONICET, the Santa Fe Agency for Science, Technology and Innovation (ASACTEI), and The Company of Biologists, for subsidizing the congress; The company Bioingeniería and the College of Physicians of Santa Fe Province, for their financial contributions and The School of Biochemical and Pharmaceutical Sciences (FbioyF, UNR), for generously yielding their space for the Teacher's Meeting.

The support for scientific excellence deserves a separate mention: I thank the Camilión de Hurtado Family for continuing to support science with the award that bears their name, and the company Inmuovi, which finances the SAFIS Award that this year we have chosen to associate with the memory of our dear member, Dr. Aldo Mottino, who passed away two years ago.

We hope this meeting serves as an excellent framework for learning, updating knowledge, and networking. You are all invited to fully enjoy this congress.

Thank you very much!



**VIII Encuentro Nacional de Docentes de
Fisiología y Física Biológica**

22 de octubre 2025

Facultad de Ciencias Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario



SAFIS

**VIII Physiology and Biological Physics
Education Meeting**

Program

14:48 -13.00	Welcome words	
13:00 – 13:40	Mini-Orals I – Curriculum Integration Strategies in Physiology Education	Presentation of experiences focused on integrative proposals within the curriculum and their application in Physiology teaching. Q&A and debate at the end of the session.
13:40 – 14:20	Mini-Orals II – ICTs and Didactic Strategies for Physiology Education	Proposals incorporating digital technologies and active methodologies to enhance teaching in diverse contexts. Q&A and discussion space with the authors following the presentations.
14:20 – 14:40	Break – Coffee Break and Informal Gathering	
14:40 – 15:30	Round Table – The Role of Teaching Assistants (Auxiliares de Segunda) in Physiology Education	Shared reflection among assistants from different academic units. Results of a national survey of teaching assistants will be presented as a starting point for discussion. Open exchange with attendees. Moderated by: Prof. Silvina Lyons, Pedagogical Department, School of Medical Sciences, National University of La Plata.
15:30– 15:45	Break – Transition to the Next Session	
15:45– 16:25	Mini-Orals III – Evaluation Practices in Physiology	Experiences related to formative, summative, and alternative evaluation strategies within the framework of Physiology education. Q&A and debate with the speakers at the end of the session.
16:25– 17:05	Reflective Opening: " Rethinking the teaching of Physiology: An integrative proposal "	Dr. Noelia Valle, Professor of Cell Biology and Physiology, Francisco de Vitoria University, Spain.
17:15– 18:45	Workshop – "Core Concepts" in Physiology Education	Presentation of results from a national survey of faculty on the concepts considered central to Physiology education. Participatory activity for collective analysis and prioritization of these concepts. Final presentation and reflection based on the work conducted in the workshop. Moderated by: Lic. Sebastián Caffera, School of Medicine and Hospital de Clínicas, University of Buenos Aires.
18:45 – 19:00	Break – Informal Closing	

Welcome words

Esteemed faculty and researchers,

For our society, research cannot be separated from teaching. This faculty gathering identifies us as a community and seeks to ensure we remain capable of transmitting our knowledge to our students. Our society and our students are constantly changing, and we must adapt to these changes. This is an opportunity for debate and for learning. The chosen setting—a Faculty of Sciences within a Public University—is the most suitable place to hold this faculty meeting.

On behalf of the SAFIS Steering Committee, I extend my warmest and sincerest thanks to the entire Faculty Committee, which has made a great effort to organize this event. We thank everyone who has made its realization possible: Dr. Valle for her lecture, the coordinators, and the participants who are presenting their experiences.

Special thanks go to the School of Biochemical and Pharmaceutical Sciences for providing us with their space to hold the meeting and to all the institutions that have endorsed this VIII Faculty Meeting.

Finally, my gratitude to all the attendees who have traveled from various parts of the country to participate in this gathering. I sincerely hope that it will be useful and beneficial for the teaching work that each of you carries out daily.

Enrique Sánchez Pozzi, President SAFIS

Conference

RETHINKING THE TEACHING OF PHYSIOLOGY: AN INTEGRATIVE PROPOSAL

Noelia Valle

Universidad Francisco de Vitoria, Madrid, Spain

Physiology is a science centered on function, on the processes that allow the organism to remain alive and stable in a changing environment. The main difficulties identified in its learning are: 1) the high cognitive load derived from complex mechanisms of homeostatic regulation; 2) a teaching approach focused on mechanisms rather than the teleological meaning of processes; and 3) the challenge of identifying and integrating core concepts that recur throughout the subject. Although the use of innovative active methodologies in recent years has partly alleviated these difficulties, we consider that a deeper transformation is required to achieve meaningful and holistic learning. Traditional university teaching reproduces the anatomical scheme, organizing content by systems which encourages mechanistic learning but hinders a global view of the organism and an understanding of homeostasis as an overarching principle.

The proposal, implemented in the Biomedical Sciences degree at Universidad Francisco de Vitoria, reorganizes the course around core concepts and the variables of the internal environment on which life depends (blood pressure, glycemia, temperature, oxygenation, acid–base balance, osmolarity.). In this way, each process is studied not as an isolated fact but as part of the network that sustains life.

The syllabus is structured into four blocks. The first addresses cellular foundations, highlighting threshold concepts such as gradients, intercellular communication, and regulatory systems, with emphasis on their elements (receptors, integration centers, and effectors). The next three are organized by functions: motor, visceral, and cognitive. In the visceral block—the most extensive—each topic focuses on a homeostatic variable, analyzing the state of normality, the causes of alteration, and the organism's compensatory mechanisms. Finally, the block on higher cognitive functions—consciousness, emotions, learning, language—approaches the brain from the perspective of the mind and its emergent properties, opening an interdisciplinary dialogue with anthropology and ethics.

The results of its implementation over two academic years show that final grades do not differ significantly from those of the traditional model. However, an evaluation conducted four months later demonstrated more lasting and integrated learning among students in the restructured course, particularly in tasks requiring the integration of knowledge from different areas.

ABSTRACTS

Curricular Integration Strategies in Physiology Teaching Estrategias de integración curricular en la enseñanza de Fisiología

DEI 01

LEARNING ABOUT FORCE & STRENGTH CONCEPTS

Borgatello C¹, Navone M³, Martínez Lotti G⁴, Wickler M².

¹ *Cátedra de Fisiología, Kinesiología y Fisiatría, Facultad Ciencias de la Salud, Pontificia Universidad Católica Argentina Sede Rosario.*

² *Cátedra de Biofísica y Director de Carrera, Kinesiología y Fisiatría, Facultad Ciencias de la Salud, Pontificia Universidad Católica Argentina Sede Rosario.*

³ *Cátedra de Informática, Kinesiología y Fisiatría, Facultad Ciencias de la Salud, Pontificia Universidad Católica Argentina Sede Rosario.*

⁴ *Decano Facultad Ciencias de la Salud, Pontificia Universidad Católica Argentina Sede Rosario.*

Introduction. Learning the concepts of force is a challenge for students who rehabilitate people. In physics, force is an agent capable of changing the shape or state of motion of a body (pushing or pulling an object with mass, changing its direction, compressing or stretching it), having magnitude, direction, and sense. In physiology, force or strength is the ability of a muscle group to generate tension against resistance by pushing, pulling, or lifting. Both are measured in Newton or kilogram-force (kgf).

Objectives. This activity had the purpose of learning and integrating the concepts of force and then assessing the handgrip strength by digital dynamometer.

Methods. A sample of 42 students (physical therapy, nutrition, and speech therapy) with a mean age of 20.8 years old (31 female and 11 male) completed the activities under the supervision of teachers. First, the students answered a trivia about physics and physiology, and then they were measured by anthropometry. Finally, handgrip strength was recorded by digital dynamometry (Vernier Inc.).

Results. The trivia about physics was answered correctly by 72.31% in physical therapy, 71.9% in nutrition, and 63.75% in speech therapy students. While physiology trivia was answered correctly by 85.33% in physical therapy, 78.26% in nutrition, and 72.5% in speech therapy students. Handgrip strength by digital dynamometry (mean \pm SD) showed that the female students recorded an average of 21.21 ± 3.73 kgf, and the male students 33.17 ± 5.92 kgf; $p < 0.0001$ by t-test. Taking into account strength in relation to body mass index (BMI), female students showed values of 0.9 ± 0.17 (BMI 23.65 ± 3.03) and male students 1.36 ± 0.21 (BMI 24.39 ± 3.19); $p < 0.0001$ by t-test.

Conclusion. This activity allowed students to understand the concept of force from different perspectives. The trivia were important in sparking curiosity and interest while providing information on basic concepts of both areas. They also learned how to correctly measure handgrip strength using dynamometry. The recorded values were consistent with those found in the studies according to age and gender.

DEI 02

ACADEMIC PERFORMANCE OF STUDENTS IN PHYSIOLOGY FROM THE CARDIOLOGICAL PRACTICES TECHNICIAN PROGRAM DURING 2023–2024.

Alcaraz Juan Ignacio, Yeves Alejandra

Cathedra of Physiology, Cardiological Practices Technician Program, Faculty of Medical Sciences, UNLP.

Introduction. The first year of university represents a substantial change in students' lives, as it requires adapting to new academic demands, more intensive study rhythms, and efficient time management. In this context, the subject Physiology, an annual first-year subject in the Cardiological Practices Technician (CPT) program, poses a major challenge in the teaching–learning process.

Objective. To analyze academic performance in the Physiology course of the CPT program at the Faculty of Medical Sciences (UNLP), with the aim of designing pedagogical strategies to improve student retention and performance.

Methodology. A retrospective quantitative study was carried out, based on data provided by the Physiology course. Partial exam results (multiple-choice written tests) and final exam results (oral assessments) from the 2023 and 2024 cohorts were analyzed.

Results. In 2023, out of a total of 543 students, 227 (56%) passed the course, 177 (44%) failed, and 139 dropped out. In 2024, out of 472 students, 121 (52%) passed, 111 (48%) failed, and 240 dropped out. Regarding final accreditation, 67 students (29%) passed in the sessions of 2023, while 82 students (67%) passed in 2024.

Conclusion. The results reveal: (I) a considerable increase in course dropout, (II) a relatively stable pass rate around 50%, and (III) a low percentage of accreditation through final exam, which may impact academic continuity. Based on these findings and surveys conducted in 2023 and 2024, potential causes of dropout were identified: more than five years between completing high school and entering university, and difficulties in time management, often associated with early workforce entry. On this basis, teaching–learning strategies are proposed to improve student retention and academic performance.

ELECTING PHYSIOLOGY: WHAT BIOTECHNOLOGY STUDENTS VALUE AND WHAT THE RESULTS SHOW US

Marrone J, Favre C

Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR.

Introduction. Physiology, as an experimental science, integrates theory, practice, and critical analysis. Animal Physiology and Biochemistry is an elective course for final-year Biotechnology students at UNR. Pasteur reveals that "the lab is the source of the strength the scientist holds in society" (Latour, 1983). To that end, despite its length (60 h), this course combines theoretical classes, exercises and an experimental lab session. The Biotechnologist profile is broad and includes health-related areas, but key courses like Physiology are elective. Enrollment has halved last year, maybe due to complex trends in academic and scientific fields. This work reflects on these academic trends and that context, and analyzes the impact of the course design through student perceptions and academic performance.

Objectives. 1. To characterize students who choose the course. 2. To evaluate how lab impacts on the students and their learning, comparing performance on topics with and without experimental support (Liver vs. Renal). 3. To assess the student rating of the course content and its future use.

Methods. A structured survey (N=21, 2018–2025) with closed and open-ended questions was used. We also compared exam scores on Liver vs. Renal topics.

Results. Motivations: Over 90% of students chose the course for its content, and 75% expressed a clear interest in biomedical topics.

Lab experience: 90% considered the lab essential or very important for gaining skills. While no significant differences were observed in exam marks in Liver vs. Renal topics, suggesting the lab reinforced general rather than topic-specific knowledge.

Short- and long-term value: Over 80% rated the content highly. Despite the enrollment drop, 54% believed the course should be mandatory. After a year or more, 66% of students reported retaining the content well, and had applied some or much of the course's framework in their work.

Conclusion. The findings highlight the value students place on the Physiology elective course. The lab is seen as essential for developing scientific skills. Results guide us to adjust the course content and also to reconsider the role of Physiology in Biotechnology curricula.

EVALUATION OF FREQUENCY, PATTERN AND ASSOCIATED MOTIVATIONS FOR THE CONSUMPTION OF ENERGY DRINKS IN A COHORT OF PHYSIOLOGY STUDENTS.

Martínez Fava Pilar¹, Iurescia Agustin¹, Assali Lucrecia¹, Toledo Dolores¹, Farela Angelina¹; Acebal Camila¹, Oca Catalina¹, Escobar Leonardo¹, Paucar Samira¹, Mazza Juan Pedro², Ennis Irene^{1,2}, Gonano Luis^{1,2}.

¹ *Cátedra de Fisiología y Física Biológica, Facultad de Ciencias Médicas UNLP.*

² *Centro de Investigaciones Cardiovasculares Horacio Cingolani, CONICET-UNLP.*

Introduction: Medical students represent a subgroup of adults with a high prevalence of energy drink consumption. However, the long-term physiological effects of chronic intake remain poorly understood.

Objectives: To determine the prevalence and patterns of energy drink consumption, to identify motivations and to explore its relationship with cardiovascular risk factors. A secondary objective is to involve teaching undergraduate assistants in scientific research to improve their teaching capacity.

Methodology: an anonymous questionnaire was distributed for voluntary participation by students. Another was distributed among teaching assistants for a preliminary evaluation of their experience.

Results: We obtained 447 responses from students reporting family history of cardiovascular diseases in 50.6 % of cases. 7.6 % of the responders had a previous measurement of high blood pressure. 35.7 % reported consumption of energy drinks, being 69.5 % sporadic. Regular consumers were grouped in 17.5 % with less than 500 ml/week, 7.8 % 500-990 ml/week and 5.2 % who drink 1 liter or more/week. The indicated amounts were sustained for more than six months at 88% and frequently mixed with alcoholic beverages at 11 %. Motivations were reported as follows (allowing for more than one option per student): 74 % to stay awake or concentrated while studying, 21.4 % to improve physical performance in sports practice, 42,9 % because of its flavor, 13,6 % to maintain active during night feasts.

In parallel, undergraduate teaching assistants mostly reported that their involvement in a departmental research project improves their capacity to analyze and communicate research results and enhances their motivation to be further involved in physiology teaching.

Conclusion: Energy drink consumption is common among our students. Although usually sporadic, more than one quarter reports sustained use. Energy drinks are widely perceived as enhancing academic performance, underscoring the need for further research. Additionally, preliminary findings suggest that involving undergraduate teaching assistants in research may positively influence their motivation and skills in teaching and research.

IMPACT OF NEW METHODOLOGICAL AND CURRICULAR APPROACH TO THE PHARMACOLOGY COURSE IN THE BIOCHEMISTRY UNDERGRADUATE PROGRAM ON STUDENTS' PROGRESS THROUGH THE COURSE.

Bulacio RP, Campagno RV, Trebucovich M, Hazelhoff MH, Brandoni A.

Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario, Argentina.

Introduction. A comprehensive curricular and methodological reorganization of the Pharmacology course in the Biochemistry undergraduate program at the Faculty of Biochemical and Pharmaceutical Sciences, National University of Rosario, has been implemented with continuous improvements from 2022 to the present. This initiative aimed to strengthen meaningful learning and promote a course approach more aligned with the future professional practice of biochemists.

Objectives. To assess the academic performance of Pharmacology students enrolled from 2023 onward, in order to determine the effectiveness of the newly implemented practices.

Methodology. Academic data from students between 2023 and 2025 were analyzed. From the total number of students per year, the percentage of those who did not meet the minimum requirements to be eligible as regular students and those who qualified for a promotional exam was estimated. In addition, the performance of students who qualified for direct accreditation exam was analyzed.

Results The percentage of students who did not meet the minimum requirements to be eligible as regular students pronouncedly decreased in 2025 compared to previous years (8.5% in 2023, 9.4% in 2024, and 3.51% in 2025). Regarding those who qualified for a promotional exam, an important increase was observed in 2024 (31.9% in 2023, 53.13% in 2024) which was remained in 2025 (49.12%). Moreover, the percentage of students who passed the promotional exam and consequently passed the course notably increased in 2024 and remained in 2025 (31.9% in 2023, 50% in 2024, and 42.11% in 2025).

Conclusions. These results demonstrate positive effects of the curricular and methodological changes on student performance and their academic progress. Evaluating the impact of the implemented changes on students will enable us to guide our teaching practices toward continuous improvement.

DEI 06

ANALYSIS OF STUDENTS' PERCEPTION OF METHODOLOGICAL AND CURRICULAR CHANGES IN THE PHARMACOLOGY COURSE OF THE BIOCHEMISTRY UNDERGRADUATE PROGRAM.

Bulacio RP, Trebucobich MS, Molinas SM, Hazelhoff MH, Brandoni A.

Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario, Argentina.

Introduction. In 2022, the Pharmacology course in the Biochemistry undergraduate program at the Faculty of Biochemical and Pharmaceutical Sciences, National University of Rosario, was reorganized to help students integrate theoretical knowledge with professional practice. The approach is based on active learning, requiring active involvement of students to integrate concepts with professional experience. To this end, problem-based learning and case study methods were applied. After four years of application, it seems valuable to analyze student perceptions of this educational innovation to identify areas for improvement and strengthen training.

Objectives. To assess student perception and satisfaction with the changes introduced in Pharmacology since 2022.

Methodology. A survey was conducted via Google Forms among students from the 2022–2024 cohorts at the end of their coursework. Responses were received from 12 students in 2022, 16 in 2023, 7 in 2024 and 22 in 2025.

Results. Most students considered the Pharmacology course aligned with the biochemist's professional profile (100% in 2022, 94% in 2023 and 100% in 2024, 96% in 2025). Classroom activities designed to apply theoretical topics were highly valued by students, with highly positive evaluations (83% in 2022, 81% in 2023, 100% in 2024, and 91% in 2025). Across all years evaluated, 100% of the students found lectures didactic and useful. Open-ended responses highlighted missing topics such as oral contraceptives, vitamins, and supplements. The majority emphasized case-based work as the most useful component. Suggestions varied, including the request in 2024 for guiding questions to support case resolution, which was adopted in the 2025 course.

Conclusion. Overall, feedback was strongly positive, underscoring the benefits of this new perspective of the course for the Biochemist's professional role. Assessing the impact of these reforms enables us to guide future practices toward continuous improvement.

Evaluation Practices in Physiology

Prácticas de evaluación en Fisiología

DEP 01

**AN APPROACH TOWARDS EDUCATIONAL RESEARCH: THE PROCESS OF
FORMULATING AN OPERATIONAL DEFINITION**

Romano Juan, Demaria Inés, Borri Octavio, Bureu Maria, De sogos Alfonso, Pereira Tauzy Justo, Schwarzstein Sara, Severini Celeste, Vidosevich María, Arias Pablo

Cátedra de Fisiología Humana, Facultad de Ciencias Médicas, Universidad Nacional de Rosario

Introduction. Within the framework of the “Undergraduate Teaching Assistant Program 2023” at the Physiology Department, Faculty of Medical Sciences (UNR), a teaching practice approach including non-participative observation of tutoring sessions in the second year of the medical career. An observation guide was designed, including “punctuality” as a variable, a factor that might negatively influence students’ performance during sessions. However, no suitable definition of “punctuality” was found in the literature. We therefore aimed firstly at developing an operational definition of this term.

Objectives. To develop an operational definition of punctuality to be applied in tutoring session observations.

Methods. Based on readings about “punctuality” and “operational definitions,” an initial draft definition was proposed: “a social and cultural agreement among the parties involved, based on respect for the scheduled starting time of an activity.”

It was defined that the beginning of the activity was determined by the tutor’s arrival, assigning a tolerance of 10 minutes to still consider the start as “punctual”. Subsequently, it was concluded that because the tutor’s arrival depends exclusively on his/her own will, the expression “social and cultural agreement” was not applicable in this context.

Results. The process led to the following operational definition of punctuality: “the tutor’s arrival at the tutorial space within 10 minutes of the scheduled starting time, thereby initiating the teaching activity.”

Conclusion. Establishing a concrete operational definition of punctuality facilitated data collection and reduced observer subjectivity.

DEP 02

OPEN BOOK FORMATIVE ASSESSMENT BASED ON FICTION NARRATIVES WITH EMBEDDED PHYSIOLOGICAL DATA

Aguilar B, Bratovich C, Menghi ML, Siebenlist M, Zapata D

Facultad de Ingeniería, Universidad Nacional de Entre Ríos, Argentina.

Introduction/Problem: For the past seven years, we have implemented a physiology content exam in the third year of biomedical engineering based on fictional narratives containing embedded data (numbers, graphs, tables, photos with inferable scale). During the exam, students may use the internet and artificial intelligence on institution provided computers (no mobile

phones). The formative assessment prioritizes the application of physiological criteria, interpretation of texts and graphics, and the identification of critical missing data for problem solving, completed with plausible assumptions consistent with the narrative.

Objectives: (1) Strengthen reasoning from text under uncertainty; (2) assess interpretation of graphs/tables and retrieval of implicit/absent data; (3) require quantitative consistency among proposed values, calculations, and embedded data; (4) specify operational conditions and scoring criteria to facilitate adoption.

Methods: Open book written assessments: students may use notes, books, the internet, and AI platforms on institutional computers. The assignment presents coherent fictional scenarios with explicit and implicit cues. Students extract and interpret information, apply physiological criteria, read graphs/tables, detect missing data and complete them with plausible assumptions, formulate hypotheses, and justify them. Validation: alignment with the syllabus, internal consistency, controlled ambiguity, and multiple solution paths.

Results: We observe improvements in reasoning from text, discrimination of pertinent information, reading of graphs/tables, appropriate use of assumptions, and greater quantitative consistency within limited time; these gains are reinforced by the expectation and motivation induced by the fictional narrative.

Conclusion: Open book assessment with fiction narratives and embedded data is suitable for evaluating data driven reasoning from text and quantitative consistency in Physiology and Biophysics.

DEP 03

IMPLEMENTATION OF THE CONCEPT NOTE IN THE CLINICAL PHARMACY SUBJECT OF THE PHARMACY DEGREE

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Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR), Rosario, Argentina.

Introduction: Clinical Pharmacy is part of the fifth year of the Pharmacy degree program at the Faculty of Biochemical and Pharmaceutical Sciences, National University of Rosario. The assessment for regularization consists of a partial exam with a retake opportunity and the presentation of a final project. New assessment strategies are necessary to more clearly represent student learning progress. The Individual Concept Sheet (ICS) is a useful tool in this teaching-learning process, which encourages active involvement from both students and teachers, enhances feedback during classes, and aims to improve student performance. Additionally, it fosters continuous assessment beyond traditional exams or oral presentations.

Objectives: To incorporate the ICS into the evaluation system for the Clinical Pharmacy subject in practical activities, offering a more comprehensive and ongoing assessment of each student.

Methodology: The ICS was designed to assess each student's listening, contribution, integration, and critical thinking. Professors in charge of each group of students had to complete after each weekly activity. Once the course ended, the results of the midterm and promotional exams, the oral presentation, and the final grade were analyzed. We compared the outcomes from the last year without the ICS (2023) to the first year with its implementation (2024).

Results: This analysis shows that in 2023, promoted students reached a percentage of 40.7, increasing to 46.9 in 2024. Furthermore, 100% of promoted students passed the promotional exam (none were absent) In contrast, in 2023 only 68.2% passed the promotional exam, with 9.1% not attending it.

Conclusion: These results emphasize the importance of implementing a concept note through a ICS. This type of formative assessment allows for individualized monitoring and promotes greater participation from the student being assessed against specific standards to be met. Ultimately, this leads to improved student performance, as students see the results achieved in a concrete way.

DEP 04

STUDENT OUTCOMES AFTER CURRICULAR REORGANIZATION IN PHARMACOLOGY I

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Introduction. In 2023 and 2024, the Pharmacology I course of the Pharmacy program (Faculty of Biochemical and Pharmaceutical Sciences, Rosario National University, Argentina) was reorganized to better align with the pharmacist's professional profile. A direct accreditation exam and a personalized evaluation sheet, used as a continuous assessment tool within practical classroom activities, were also introduced, allowing detailed monitoring of each student's progress and individual learning needs. These changes aimed to foster meaningful learning and support the transfer of knowledge to professional practice. Evaluating their impact is essential to determine effectiveness and guide future improvements.

Objectives. Analyze the academic performance of Pharmacology I students between 2023 and 2024 in order to evaluate the impact of the implemented curricular and methodological reorganization.

Methodology. Students were classified into four categories: *undergoing direct accreditation* (P), *fulfilled course requirements* (R), *not qualified* (F), and *discontinued the course* (D). The

performance of P students was also examined. Results were expressed as the number of students and percentages.

Results. In 2024, an increase in the proportion of P students was observed compared to the previous year (29% in 2024 vs. 12% in 2023). Additionally, there was a decrease in D students (2% in 2024 vs. 14% in 2023), while the proportion of F students remained largely unchanged (22% in 2024 vs. 24% in 2023). Among P students, exam success rates were similar (88% in 2024 vs. 86% in 2023).

Conclusions. These results show an increase in the number of students taking the direct accreditation exam and a decrease in those who discontinue the course, suggesting that the curricular reorganization of the Pharmacology I course and the implementation of the individualized continuous assessment tool contributed to improved student persistence and performance.

DEP 05

PHYSIOLOGY ASSESSMENT FOR BIOCHEMISTRY AND PHARMACY: RELIABILITY ANALYSIS

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Introduction/Problem: Over the past two years, the evaluation of students in Human Physiology at Universidad Nacional del Sur (UNS) has been summative, considering student participation, quiz scores (five quizzes before the midterm exam), and written exam scores (two exams during the course). Although innovations have been implemented to evaluate different aspects of student performance and to establish a continuous assessment approach, no study has been conducted to examine the validity and reliability of these assessments in education.

Objectives: The objective was to analyze the reliability of written midterm exams by conducting correlation studies between question types and student scores, in order to determine whether the different question styles effectively discriminate among students with varying levels of knowledge and skills.

Methodology: Data were collected from 72 students in 2023 and 97 students in 2024 who completed the physiology exam that included:

- **Context-based open-ended questions** (e.g., clinical cases or physiological regulation scenarios): designed to deeply assess specific knowledge and skills.
- **Context-free multiple-choice questions:** designed to assess general knowledge in a more superficial manner.

The correlation between scores obtained for each question type and the students' final exam grades was calculated, categorizing grades into four ranges: 0–25, 25–50, 51–75, and 76–100.

Results: The results showed that context-based open-ended questions were statistically significant direct correlation with student grades across all four ranges ($p < 0,01$). However, for context-free multiple-choice questions, a statistically significant direct correlation was observed only in the 0–25 ($p < 0,01$) and 76–100 grade groups ($p < 0,01$), with no correlation found in the 25–50 and 51–75 groups.

Conclusion: The findings suggest that context-based open-ended questions are a more reliable predictor of student performance in physiology than context-free multiple-choice questions. Incorporating context-based open-ended questions can enhance the effectiveness of assessing students' specific knowledge and skills. It is crucial to review and improve assessment items to ensure their reliability in measuring performance.

ICTs and Teaching Strategies for Physiology Education

TICs y estrategias didácticas para la enseñanza de la Fisiología

DTIC 01

A HANDS-ON EXPERIENCE: AN UNIQUE AND INTEGRATIVE TEACHING APPROACH TO PHYSIOLOGY

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Introduction Practical lessons (PLs) with teaching assistants (TAs) are a key part of Physiology education, yet students' experiences during these remain underexplored. During the first quarter of 2025, a new approach incorporating three integrative assignments (IAs) was introduced to the teaching of physiology for Pharmacy and Biochemistry students at FFyB-UBA. Understanding how students interact with PLs, IAs and related materials is crucial to improve future teaching strategies.

Objectives To evaluate the organization, development, and perception related to students' experience during PLs with TAs. The study also aimed to assess the use of didactic materials and differences between first-time and repeating students, the number of IAs and overall course design.

Methods A survey containing both closed and open-ended questions was answered by 27 students. Their demographics (age, degree, employment status, first-time vs. repeating students, final course status), use of learning resources (virtual campus materials, textbooks, lectures), and

engagement strategies were assessed. Differences between morning and evening student groups were also considered.

Results Findings showed high acceptance of the new Physiology teaching format. Most students rated its organization and teaching positively, and almost all valued the integration of theory and practice. Multiple IAs were preferred to a single final one, and the order of units was considered useful. Students academic background seemed to influence their performance. End of class summaries by TAs were described as very useful for consolidating knowledge. Qualitative feedback emphasized motivation, teamwork, and better understanding, although some noted time pressure and requested fewer non-essential exercises. Overall, the format was perceived as innovative, engaging, and effective in promoting meaningful learning.

Conclusion Students' experiences are influenced by academic background, resource use, and engagement with new teaching tools. The incorporation of IAs provided an innovative and effective way to improve comprehension and participation during Physiology PLs, as students responded positively to them. Further improvement is necessary to better the teaching and integrative experience.

DTIC 02

IMPLEMENTATION OF FLIPPED CLASSROOM IN THEORETICAL PHYSIOLOGY CLASSES IN THE BIOCHEMISTRY SCHOOL AT THE UNIVERSIDAD NACIONAL DE ROSARIO: IMPACT ON STUDENT PERFORMANCE

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Introduction. Until 2023, the topics *Excitable Tissues* (ET) and *General Mechanisms of Hormones* (GMH) were taught exclusively through pre-recorded theoretical videos plus traditional lectures. To promote meaningful learning, in 2024 we redesigned theoretical classes for these topics using a flipped classroom approach, incorporating problem-solving activities as triggers for discussion, peer interaction, and teacher–student dialogue.

Methods. We conducted a longitudinal analysis comparing student performance in ET and GMH between the 2023 and 2024 cohorts. Scores were calculated as the fraction of points obtained in each question relative to its total value, normalized to the student's exam grade. This score reflects the relative contribution of each specific topic to the exam grade for each student. For ET, *Renal Physiology* (RP)—a topic with unchanged teaching methods between 2023 and 2024 and evaluated in the same exam as ET—was used as an internal control which is not biased by the contribution of ET points to the exam grade. ET performance was therefore expressed as

ET/RP ratios. Results were analyzed using unpaired t-tests and expressed as mean \pm SD ($p < 0.05$). The same teaching staff was responsible for ET, GMH, and RP in both years.

Results. GMH showed a significant improvement (GMH2023: 0.71 ± 0.38 ; GMH2024: $1.37 \pm 0.29^*$). For ET, scores alone did not differ significantly (ET2023: 0.65 ± 0.41 ; ET2024: 0.68 ± 0.45), but improvement emerged when normalized to RP (ET/RP2023: 0.64 ± 0.54 ; ET/RP2024: $0.96 \pm 0.85^*$). Overall performance, assessed as the percentage of students who achieved course regularity in Physiology, was slightly higher in 2024 than in 2023 (70% vs. 65%, respectively).

Discussion and Conclusion. Implementing a flipped classroom strategy substantially enhanced student performance in GMH, assessed as GMH contribution to the overall student performance. In ET, improvement was evident when normalized to an internal control. These results suggest that adopting flipped classroom approaches in theoretical modules not traditionally taught through active learning in our course is associated with measurable academic benefits.

DTIC 03

INSECTS AS AN ALTERNATIVE MODEL FOR STUDYING THE HEART PROPERTIES AND FUNCTIONING OF CIRCULATORY SYSTEMS ON ANIMAL PHYSIOLOGY SUBJECT.

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Introduction: In physiology the understanding of circulatory system is improved when students can visualize experimentally how it functions. Classically, these experiments were conducted using vertebrates. However, in the last years its use for educational purposes has been ethically questioned by experts on animal care, and also by students, who express disagreement with these kinds of practices. Additionally, its use involves high costs and requires expertise for the manipulation. Altogether, these facts raise the need to think of new pedagogical strategies and experimental tools in our course.

Objective. To test a model based in the insect *Rhodnius prolixus* for the study of circulatory system in practical classes.

Methods. We adapted a protocol previously developed for research on the laboratory of Animal Histology and Embryology (FCNyM-UNLP). To study the functioning of the insect “heart” we used unfed adults of *R. prolixus*. They were placed on a petri dish with paraffin and held with plasticine. The transparent cuticula allows the direct view of the dorsal vessel (aorta) and the anterior midgut which also participates in circulation. The number of dorsal vessel and crop contractions in a 3 min period were registered under resting conditions. Then, in order to observe the regulation of

the frequency of contraction chemical messengers were applied (5-HT and Ach). Finally, we analysed the data obtained, and compared to other animal groups. In addition, the students observed how the circulation occurs in an open circulatory system.

Results. The protocol resulted in a good model to study the basic functioning of the circulatory system. The results can be easily extrapolated to other animals. It allowed the students to understand how this system functions, and how it is regulated by nervous and endocrine signals. Additionally, this model allows a comparative analysis with vertebrate's system.

Conclusion. This activity was a positive pedagogical experience enabling the study of basic and comparative characteristics of circulatory systems. Despite we still used animals for teaching, it was better accepted by students and ethical committee.

DTIC 04

DIGITAL GAME-BASED LEARNING IN PHYSIOLOGY: STUDENT ENGAGEMENT WITHOUT MEASURABLE KNOWLEDGE GAINS

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Background: One of the current challenges in physiology teaching is addressing the learning profile of digital natives, whose cognitive approaches differ from previous generations. According to Prensky, these students (i) are less engaged by unidirectional lectures, (ii) are strongly inclined to use contemporary technologies, and (iii) value both cooperation and competition. Digital game-based teaching has been shown to foster active learning by enhancing motivation, participation, and the acquisition of cognitive and technological skills.

Objective: To evaluate the impact of incorporating a digital game into lectures on *Female Sex Hormones* (FeSH) on student learning perceptions and outcomes.

Methods: The study was conducted using a non-experimental design with qualitative and quantitative approaches. A serious [mini-escape room game](#) was developed in *Genially* and integrated as a group activity during the 2024 FeSH lecture. Game participants completed a survey rating the experience (poor–excellent) and its contribution to understanding (yes/no/not sure). Academic performance was assessed through the FeSH exam question comparing: (i) game participants (G, n=15) vs. non-participants (Control, n=30) in 2024, and (ii) the relative contribution of FeSH to exam grades between 2023 (n=47) and 2024 (n=45) cohorts (t-test, mean \pm SD).

Results: Among the G group, 50% rated the experience as *very good* and 50% as *excellent*; whereas they all reported that it supported their understanding. However, exam performance in FeSH did not differ between groups (Control: 69% \pm 23; G: 67% \pm 24), neither did the 2024 cohort

show an improvement in the FeSH performance compared to the 2023 cohort (2024: 1.2 ± 0.4 ; 2023: 1.1 ± 0.3).

Discussion/Conclusion: Incorporating a digital escape room game into the FeSH lecture was highly valued by students, although no measurable improvement in exam performance was observed. Gamification may still confer additional benefits —such as increased motivation, engagement, or transversal skill development— that were not assessed here. These findings also highlight the need for caution when interpreting perception data as a surrogate for actual learning outcomes.

DTIC 05

PEDAGOGICAL EXPERIENCE: INVERSE PHYSIOLOGY FROM FILM SCENES IN A BIOMEDICAL ENGINEERING PHYSIOLOGY COURSE

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Introduction/Problem: Cognitive and technological shifts (ubiquitous information, AI tools, multitasking) call for teaching centered on effect→cause reasoning. This experience starts from observable cues in film scenes and invites inference of the underlying physiological processes. Films were also vetted for human values (ethics, empathy, solidarity), enabling brief exchanges without displacing the technical focus.

Objectives: (1) Shift emphasis from memorization to modeling and critical judgment; (2) offer a low-cost, easily replicable protocol; (3) document feasibility and perceived gains in engagement and transfer.

Methods: Over two weeks (flipped classroom with problem- and case-based activities), students worked with several scenes per prompt. The teaching team applied a Didactic Invertibility Index (DII) as a relevance filter (signal clarity, interpretable scale, recognizable temporality, stimulus–response relation, and variability). Groups delimited fragments and identified key events. Using mobile apps or custom scripts, they performed frequency analysis of sound with estimates of relative intensity, estimates of lighting change, timing and stimulus–response latencies, and geometric scale estimation to derive pertinent magnitudes. Assumptions were reported; inferences were reviewed through simple tests, alternative measurements, and objective external references. Deliverables: a traceable, coherent, justified report and its oral defense.

Results: We observed sustained engagement, stronger argumentation centered on estimates and uncertainty, and improved theory→context transfer (e.g., pupillary adaptation, auditory thresholds, reflex latencies). Working with multiple scenes favored comparison and verification; reflections on values were brief and pertinent.

Conclusion: Inverse physiology with film scenes is feasible, motivating, and aligned with Biomedical Engineering competencies. It strengthens effect→cause reasoning with accessible tools and is ready for broader adoption; upcoming cohorts will incorporate formal metrics and controlled comparisons.

DTIC 06

EXTRACURRICULAR COURSES: WHAT DO PHYSIOLOGY STUDENTS SEEK AND GAIN? CONTRIBUTIONS TO IMPROVING UNIVERSITY TEACHING

Perucho Benjamín, Crocci Cecilia Anabel, Becerra Juliana, Lerda Soriano María Dolores, Gasparini María, Saavedra Amparo, Di Biasi Violeta, Ennis Irene Lucía, Ibáñez Alejandro Martin
Cátedra de Fisiología y Física Médica, Facultad de Ciencias Médicas, UNLP.

Introduction. Currently, it is frequent for students to enroll in paid courses to prepare for their subjects. Presumably the reasons for doing it may include limited class hours and the need to gain confidence for oral examinations.

Objectives. To survey physiology students at the Faculty of Medical Sciences, UNLP, regarding their participation in extracurricular courses, underlying motivations, and perceived benefits, with the aim of identifying potential improvements in teaching. To compare the academic performance of students who attend such courses with those who do not.

Methods. An anonymous survey was administered to class students of the 2025 cohort.

Results. A total of 77.2% of respondent students reported attending at least one extracurricular course, while 22.8% did not, with half due to financial reasons. The main motivations included gaining confidence, saving time and the perception that regular classes were insufficient. Only 26% enrolled after failing an exam. Reported benefits were increased confidence, better preparation prior to classes, and improved performance in oral examinations. Students also valued access to recorded lessons of higher quality or shorter duration, which were considered more useful than the department's videos. Students who attended extracurricular courses achieved higher grades in the first midterm test, while non-attendees had a higher failure rate (35% vs. 19%, $p < 0.05$, Chi-square test).

Conclusion. Participation in extracurricular courses is widespread, with financial barriers being the main reason for non-attendance. These courses play a preventive role, driven by the pursuit of confidence, security, and prior practice before classes. Academic outcomes were superior among students who attended courses. It is therefore recommended to implement additional opportunities for oral practice within regular classes, as well as through dedicated workshops or seminars. Updating the department's audiovisual resources is also suggested, prioritizing shorter video formats to enhance engagement and effectiveness.

USE OF SOCIAL MEDIA FOR MEDICAL INFORMATION AMONG MEDICAL STUDENTS

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Introduction: In the past few years, social media and artificial intelligence (AI)-based tools have become a quick and accessible source of medical information for undergraduate medical students. AI has opened a new scenario that allows immediate answers to clinical or academic questions, serving as a valuable resource to complement learning and ongoing training. However, concerns persist regarding the spread of misinformation, the superficial treatment of content, and difficulty in distinguishing reliable from unreliable sources. **Objectives:** To provide evidence and analyze the practices of medical information consumption and verification on social media among medical students.

Methodology: A descriptive, observational, and cross-sectional study with a qualitative-quantitative approach was conducted to evaluate the consumption of medical information among students from all academic years. A voluntary and anonymous 20-question survey was administered over one month, disseminated through FFB communication channels.

Results: The survey was answered by 573 students (60% were second year-students), with a mean age 23.5 years (75.0% female, 24.5% male, 0.5% not declared). Of the respondents, 45.9% reported consuming medical content on social media several times a day, with Instagram being the most used platform, followed by TikTok, YouTube, and X. Only 23.5% consistently verified the accuracy of sources. The most frequently used verification tools were Google or similar search engines (56%), AI tools (55%), textbooks or lecture notes (53%), and consultation with professors (37%). The most commonly used AI platforms were ChatGPT and Google Gemini.

Conclusion: Although social media and AI represent easily accessible educational resources, a low frequency of systematic verification of information was observed, which facilitates the spread and use of unverified content. Hence, promoting research that explores the potential negative impact these practices may exert on learning quality, the development of critical reasoning, and decision-making, while simultaneously reinforcing education on the use of tools that enable rigorous data verification, is essential.



Annual Meeting of the
Argentine Society of Physiology

PROGRAM

Wednesday October 22, 2025

School of Biochemistry and Pharmaceutics (UNR) – Suipacha 531 – Rosario

19:00- 20:30 Roundtable on Science Policy

Presenters: Dr. Claudia Capurro, CONICET; Dr. Erica Hynes, Secretary of Science, Technology, and Innovation of the Province of Santa Fe, Lic. Franco Bartolacci, rector of the National University of Rosario and vice president of CIN and Dr. Guillermo Labadie, director of CCT-Rosario

Moderator: Dr. Andrés Sciara, dean of the School of Biochemical and Pharmaceutical Sciences

Thursday October 23, 2025

Plaza Real Suites Hotel – Santa Fe 1632 Rosario

8:30 – 10:00 Cardiovascular Physiology Symposium.

"The heart in Focus: Structural Insights, Microbial Influence, and Therapeutic Advances."

"Multifaceted Functions of Junctophilin-2 in the Heart". Dr. Long Sheng Song, Edith King Pearson Chair in Cardiovascular Research, University of Iowa.

"Opportunities for harnessing the gut microbiome for cardiovascular health". Dr. Francine Marques, Victorian Heart Institute, Biomedical Discovery Institute, Monash University.

"Therapeutic Gene Transfer in the Heart Using Adeno-Associated Viral Vectors". Dr. Alejandro Orłowski, Centro de Investigaciones Cardiovasculares, Universidad Nacional de La Plata-CONICET.

10:00-12:00 Mini-orals

Salón Belgrano: Cardiovascular physiology and Hipertension – Respiratory physiology

Salón Plaza Real: Gastrointestinal physiology

12:00-13:00 Lunch Break

13:00-14:30 Gastrointestinal Physiology Symposium.

"Novel Mechanisms in Liver Disease Pathogenesis"

"Involvement of bone morphogenetic proteins in the progression of chronic liver diseases"
Agueda Gonzalez Rodriguez PhD. Institute of Biomedical Research Sols-Morreale (CSIC-UAM), Madrid, Spain. Network Biomedical Research Center for Diabetes and Associated Metabolic Diseases (CIBERdem, ISCIII), Madrid, Spain.

“Role of Acyl-CoA Binding Protein in fatty liver disease progression: An emerging therapeutic target?” Omar Motiño PhD. Distinguished Research Scientist Departamento Bioquímica y Biología Molecular y Fisiología / Facultad de Medicina, Universidad de Valladolid, España

“Liver sinusoidal endothelial cells dysfunction in the progression of MASLD” Nadia Ciriaci, Centre de Recherche sur Inflammation, Paris, Francia

14:30-16:00 Extracellular Vesicles Symposium

“Extracellular Vesicles in Physiological and Pathological Conditions”

“Extracellular vesicles: key drivers of neuronal differentiation and plasticity”. Dra. Claudia Banchio: Laboratorio de Biología Molecular y Celular de Lípidos, Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Departamento de Ciencias Biológicas, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina.

“miRNAs-Set of Plasmatic Extracellular Vesicles as Novel Biomarkers for Hepatocellular Carcinoma Diagnosis Across Tumor Stage and Etiologies.” Dr. Luis Castro-Sánchez. Centro Universitario de Investigaciones Biomédicas, SECIHTI, Universidad de Colima, México.

“Total and Extracellular Vesicle cAMP Contents in Urine Are Associated with Autosomal Dominant Polycystic Kidney Disease (ADPKD) Progression.” Dr. Pablo Javier Azurmendi, Div. Nefrología Experimental y Bioquímica Molecular, Instituto de Investigaciones Médicas Alfredo Lanari, Facultad de Medicina, Universidad de Buenos Aires; IDIM UBA-CONICET. Buenos Aires, Argentina.

16:00-16:15 Coffee Break Salon Plaza Real

16:00-18:00 Mini-orals

Salón Belgrano Renal Physiology

Salón Plaza Real: Endocrinology – Metabolism - Reproduction

18:00-19:00 Opening Conference.

“Novel therapies for urea cycle disorders: from understanding metabolic pathways to proof-of-concept studies”. Dr. Leandro Soria, Telethon Institute of Genetics and Medicine (TIGEM). Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy.

19:00-19:30. Former President Tribute.

“Dr. Mario Parisi and the re-foundation of Society of Physiology.” Dr. Roxana Toriano. IFIBIO (UBA-CONICET)

19:30-20:30 SAFIS General Assembly

Friday October 24, 2025

Plaza Real Suites Hotel – Santa Fe 1632 Rosario

8:30 – 10:00 Camilión de Hurtado Award

10:00-11:30 Young Investigators Symposium

“Short talks: a space for YOUTH, by YOUTH”.

11:30-12:30 Lunch break – Young investigators Commission Assembly – Salón Plaza Real

12:30-14:00 SAFIS award “Dr. Aldo Mottino” sponsored by Inmuovi

14:00-15:30 Oncology Symposium

“Inside the Cancer Cell: Pathway Disruption, Nuclear Armor, and Sensory Loss.”

“From Computer to the Lab: Rac1 Pathway in 5-FU-Resistant Colorectal Cancer” Luciano Anselmino, PhD. Postdoctoral Fellow CIC-R. Facultad de Medicina, UNR. Centro de Investigación del Cáncer de Rosario. Santa Fe, Argentina.

“Nuclear Envelope in Pancreatic Cancer: the Armor Protecting KRAS Oncogenic Function”

Martin E. Fernandez-Zapico, MD. Consultant, Division of Oncology Research, Department of Oncology. Consultant, Division of Gastroenterology and Hepatology, Department of Internal Medicine. Consultant, Department of Cancer Biology. Professor of Pharmacology. Professor of Medicine. Mayo Clinic Rochester, MN, USA.

“Decoding the Primary Cilium: A Key Player in Cholangiocarcinoma Pathogenesis” Sergio A.

Gradilone, PhD. Professor and Section Leader. Cancer Cell Biology and Translational Research. Assistant Director for Education. The Hormel Institute. Masonic Cancer Center. University of Minnesota, Austin, MN, USA. 15:30-17:30

15:30-15:45 Coffee Break – Salón Plaza Real

15:45-17:30 Mini-orals

Salón Belgrano: Oncology – Genetics and Gene Therapy – Immunology–

Salón Plaza Real: Cell Physiology -Cell Signaling

17:30-19:00 Endocrinology Symposium

“The Skeletal Muscle: a new endocrine organ?”

“Recovery of Motor Function Following Recombinant Netrin-1 Administration in a Chronic Experimental Model of Severe Spinal Cord Injury”. Dr. Ramiro Quintá; Investigador

Independiente del CONICET. Laboratorio de Medicina Experimental "Sección Neuroreparación"
- Departamento de Investigación - Hospital Alemán – CABA.

"The Role of Muscle in Respiratory and Cardiometabolic Rehabilitation". Lic. Mauricio Miguel;
Kinesiología y Fisiatría UNSAM - MP 635; Esp. de Kinefisiatría Respiratoria Crítica (SATI -
UNSAM); Diplom. en Rehabilitación Respiratoria (UNSAM) - MN 8897; Kinesiología Crítica
Sanatorio Británico, Rosario; Coord. C.K.C Centro de Rehabilitación Cardiopulmonar; Miembro
Asociación Argentina de Medicina.

"A Focus on Its Cardiometabolic Function". Prof. Cristina Arranz; Profesora Titular Consulta de
Fisiología, Facultad de Farmacia y Bioquímica, UBA; Investigadora del IQUIMEFA (UBA-
CONICET). Skeletal Muscle Revisited

19:00:20:00 Closing Conference

*"The role of Metformin in Alzheimer's disease. Experimental evidence and information from a
global patient database"* Dra. Flavia Saravia, IByME-CONICET, Buenos Aires.

20:00-20:30 Awards Ceremony.

Opening Conference

NOVEL THERAPIES FOR UREA CYCLE DISORDERS: FROM UNDERSTANDING METABOLIC PATHWAYS TO PROOF-OF-CONCEPT STUDIES

Leandro R. Soria

*Telethon Institute of Genetics and Medicine (TIGEM). Via Campi Flegrei 34, 80078 Pozzuoli
(NA), Italy.*

Waste nitrogen is converted into urea by the healthy liver. Impaired ammonia detoxification leads to hyperammonemia, a life-threatening condition resulting in detrimental effects on the central nervous system. Systemic ammonia is elevated in patients with inherited urea cycle disorders (UCD), a group of inborn errors of metabolism. Because of their prevalence, severity and lack of effective treatments, novel and better therapies for UCD are needed. In this talk, I will discuss proof-of-concept data supporting the efficacy of small molecule drugs for hyperammonemia and UCD. Moreover, as UCD are complex diseases, special attention will be given to new pathogenic mechanisms leading to chronic hepatocellular injury, a common complication in patients with argininosuccinic aciduria, the second most frequent UCD. These findings illustrate how the development of novel therapies for rare genetic conditions can lead to treatments for metabolic alterations occurring in more common multifactorial disorders.

Closing Conference

THE ROLE OF METFORMIN IN ALZHEIMER'S DISEASE. EXPERIMENTAL EVIDENCE AND INFORMATION FROM A GLOBAL PATIENT DATABASE.

Nicolás Gonzalez-Perez, Carlos Pomilio, Juan Beauquis & Flavia Saravia

*Neurobiology of Aging Lab, IByME CONICET and Dept Biological Chemistry, Faculty of Exact &
Natural Sciences, University of Buenos Aires, Argentina.*

Alzheimer's disease (AD) is characterized by the progressive cognitive decline in multiple domains. Patients with type 2 diabetes mellitus (DM) have an increased risk of AD, sharing some pathogenic conditions with AD subjects, such as brain insulin resistance. For this reason, it has been anticipated that anti-diabetic medication would provide benefits against the pathogenesis of AD, often referred to as 'type 3 DM'. Metformin (MetF) is a first-line medication for DM and is among the most promising candidates for drug repurposing toward AD treatment. Therefore, our objective was to evaluate the therapeutic potential of MetF in experimental models of Alzheimer's disease and patients recruited to an observational study. Transgenic PDAPPJ20 mice at 9 months of age were treated with MetF during 3 weeks. We found a better cognitive performance and a diminished amyloid load in the hippocampus, together with a neurogenic capability recovery.

Neuroinflammatory status- including microglia reactivity- was decreased. An *in vitro* approach exposing microglial cells to amyloid peptides allowed us to evaluate the potential effect of MetF on amyloid uptake, autophagy and degradation by this crucial brain cell type. MetF was associated with a promotion of phagocytosis, amyloid degradation and reversion of amyloid-induced impairment in autophagic flux.

Using the ADNI global database, we selected patients with mild cognitive impairment (MCI) due to Alzheimer's who were being treated with MetF for their diabetes. We conducted a principal component analysis to evaluate the distribution of patients on MetF compared to the MCI population. Additionally, a paired analysis of the patients was performed (matched by sex, age, years of education, and ApoE genotype). MCI-MetF patients showed better cognitive performance than MCI patients, and these patients also exhibited a healthier biomarker profile in the CSF (A β , pTau). In the paired analysis, we also observed an improvement in MCI-MetF patients compared to other diabetic MCI patients, both in cognitive performance and in better-preserved neuroanatomical measures.

Together, our results from *in vivo* and *in vitro* AD models and the analysis of data from DM patients in the initial phase of AD, suggest a protective role for MetF in this neurodegenerative disease.

Round Table of Science Policy

CONICET IN CHALLENGING TIMES: ACHIEVEMENTS IN MANAGEMENT AND FUTURE DIRECTIONS

Claudia Capurro

*Vice President of Scientific Affairs of the National Scientific and Technical Research Council
(CONICET)*

The current context of the Argentine scientific system has been marked by significant economic and social difficulties that have threatened the continuity of public policies in science and technology. Within this scenario, our primary management goal was to keep CONICET functioning and to secure the continuation of its regular calls and activities, which are essential for the stability of the system. Thanks to the joint effort of the institution and the scientific community, it was possible to sustain doctoral and postdoctoral fellowship competitions, calls for entry into the Research Career, regular evaluation processes, and support for research projects. Preserving these instances, far from being a mere administrative exercise, represented a strategic action: it enabled thousands of young researchers and established groups to continue with their work plans, avoided interruptions in the training of human resources, and maintained the institutional framework that ensures knowledge production in the country.

These achievements testify to the resilience of Argentine science and highlight the importance of having an institution capable of resisting and adapting even in times of crisis. However, they also make clear the challenges ahead. The sustainability of the scientific system requires predictable funding and long-term policies that transcend political cycles. In particular, it is crucial to strengthen basic science as a driver of innovation and critical thinking, to guarantee conditions that allow young researchers to develop their careers in the country, and to promote internationalization and interdisciplinary cooperation as ways to enhance the impact of knowledge produced in Argentina.

In this presentation, I will share the recent management experience at CONICET, emphasizing both the actions undertaken to sustain scientific activity in an adverse scenario and the strategic directions that should guide the future. Argentine science has shown a remarkable capacity to generate high-quality knowledge even under difficult conditions; the challenge now is to transform that resilience into a solid basis for sustainable development, linking scientific production with social and economic needs while preserving the intrinsic value of knowledge as a public good.

SANTA FE: A LEADER CASE IN SUBNATIONAL POLICIES FOR SCIENCE, TECHNOLOGY AND INNOVATION

Erica Hynes

*Secretary of Science, Technology and Innovation, Ministry of Productive Development,
Government of the province of Santa Fe*

Santa Fe province has a robust innovation ecosystem, characterized by companies and startups based on technology, as well as universities and organizations in the fields of science and technology. The sub-national policies for the sector have accompanied this development. In 2008, Santa Fe assigned ministerial hierarchy for the area, and in 2013, a provincial agency for Science, Technology and Innovation (STI) was created. In 2018, Provincial Law 13742 imposed a mandatory budget for STI policies and consolidated the agency's status. In its actual configuration, the area STI is included in the Ministry of Productive Development. The recently passed constitutional reform has stated science, technology and innovate on as a public good to be protected and improved, established the right of citizens of Santa Fe to use the benefits of science and the novel knowledge created in the province, and also ordered the strengthening of the regional innovation system.

The public policies for STI in Santa Fe account for classic and novel instruments. As for classic tools, annual calls for oriented investigation in universities or research institutes are granted, as well as calls for innovation projects in the productive sector. Other programs address scientific, technological and industrial culture. They include project calls for the production of artistic and

interactive content, and territorialized projects in alliance with local governments, science clubs, and innovation platforms.

Santa Fe province designed and runs the “Centro Educativo, Científico y Tecnológico Acuario del Río Paraná”, a high-quality space aimed at science production, environmental education and conservationism, ecotourism and technological assistance to aquaculture production. Likewise, the subnational government participates in the governance of most of Santa Fe's innovation platforms. Santa Fe has also pioneered gender and sex analysis in policies of STI, with its program dated 2018, aimed at talent, careers, and leadership of women and diversity. Today, the program is focusing on producing innovation and knowledge on the subject of society and the economy of care. The next innovative policy in Santa Fe province will be the tool “Catalizar” a second grade fund addressed to innovation managers, which will distribute public funding for strategic startups through public competition.

To summarise, Santa Fe deploys a relevant portfolio of instruments of public policy for science, technology and innovation with an increasing formal framework and real impact. These are embedded in an ecosystem of strong public-public and public-private coordination, giving strength to the regional innovation system.

Franco Bartolacci

Rector of the National University of Rosario – Vicepresident of CIN

Abstract pending

SCIENCE, TECHNOLOGY AND INNOVATION IN THE TERRITORY: THE ROLE OF CONICET REGIONAL CENTERS

Guillermo Labadié

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The network of CONICET Science and Technology Centers (Centros Científicos Tecnológicos CCTs) across Argentina provides a distinctive model for decentralized scientific administration, enabling the articulation of national policies with regional dynamics. The Directors' Network of CONICET CCTs has strengthened collective strategic planning, promoting coordination and knowledge exchange among centers that operate within diverse territorial realities. Within this framework, CONICET Rosario plays a central role as a hub of scientific excellence and territorial integration in the region.

Our institutional vision of “science in the territory” is based on the active participation of all CONICET personnel — including researchers, technicians, and fellows both within institutes and across the broader zone of influence — fostering collaboration that transcends institutional boundaries. This comprehensive view aims to recognize and connect the full human and infrastructural potential of the local scientific ecosystem.

CONICET Rosario maintains a strong partnership with the National University of Rosario (UNR) and other local actors such as INTI, DAT, INTA, consolidating an open and collaborative innovation environment. These efforts are aligned with the Science, Technology and Innovation policies of the Province of Santa Fe, which emphasize federalization, sustainability, and productive transformation.

Our strategic vision includes the integration of the CCT with other components of the regional science and technology system, and the development of new strategies for the institutional visibility of CONICET. These involve not only communication and outreach but also the construction of shared spaces for co-designing projects, identifying opportunities, and strengthening the public value of scientific research. From Rosario’s experience, we underscore that genuine territorial science emerges from cooperation, diversity of perspectives, and sustained engagement with local needs and challenges.

Cardiovascular Physiology Symposium

"The heart in Focus: Structural Insights, Microbial Influence, and Therapeutic Advances."

MULTIFACETED FUNCTIONS OF JUNCTOPHILIN-2 IN THE HEART

Long Sheng Song,

Edith King Pearson Chair in Cardiovascular Research, University of Iowa.

Abstract pending

OPPORTUNITIES FOR HARNESSING THE GUT MICROBIOME FOR CARDIOVASCULAR HEALTH

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Cardiovascular disease and stroke account for 32% of all deaths globally. A key risk factor for these diseases is high blood pressure. Diet is an essential player in the prevention of these

diseases – for example, diets high in salt are associated with higher blood pressure, while diets high in fibre are associated with lower blood pressure. Besides decades of clinical and epidemiological evidence, the mechanisms driving the association between dietary fibre and lower cardiovascular disease and stroke rates remained unclear. Our research has pioneered the exciting concept that dietary fibre protects against these diseases by manipulating the gut microbiota. Here, we will present key evidence from a combination of experimental and human studies of how gut microbiota-derived metabolites called short-chain fatty acids (SCFAs) facilitate gut-to-host communication that affects the immune system and increases blood pressure. These new mechanisms, primarily regulated by two classes of G-protein-coupled receptors, represent novel treatment targets for hypertension and associated diseases.

THERAPEUTIC GENE TRANSFER IN THE HEART USING ADENO-ASSOCIATED VIRAL VECTORS

Alejandro Orłowski

Centro de Investigaciones Cardiovasculares “Dr. Horacio E. Cingolani” (CONICET-UNLP)

Gene therapy based on adeno-associated viral (AAV) vectors is rapidly emerging as one of the most powerful approaches in modern medicine. Several AAV-based products have already been approved for human use, including therapies for spinal muscular atrophy and hemophilia, clearly demonstrating that this strategy is safe, well tolerated, and capable of achieving long-lasting gene expression after a single administration. Among the different serotypes, AAV9 has proven particularly valuable for cardiac applications due to its strong tropism for cardiomyocytes and ability to efficiently transduce the myocardium following systemic delivery. These unique properties place AAV vectors at the center of innovative strategies to modulate cardiac signaling and remodeling in vivo. Despite these advances, their therapeutic potential in cardiovascular disease remains underdeveloped compared to other fields, highlighting an urgent need to generate preclinical data supporting their application in heart disease. In our lab, we applied cardiotropic AAV9 vectors to investigate and therapeutically manipulate the role of IRBIT (inositol 1,4,5-trisphosphate receptor binding protein released with IP3) in cardiac hypertrophy. IRBIT expression was detected in atrial and ventricular cardiomyocytes, fibroblasts, and human myocardium, with increased levels observed in hypertrophic mouse models and in human samples from dilated and ischemic cardiomyopathy. AAV9-mediated overexpression of IRBIT induced pathological remodeling, including ventricular dilation and impaired cardiac function, whereas long-term effects were also observed after neonatal administration. Importantly, targeted downregulation of IRBIT via AAV9-shIRBIT in transverse aortic constriction (TAC) mice prevented hypertrophy development, while silencing in sham-operated controls produced no adverse effects. These findings identify IRBIT as a novel mediator of maladaptive remodeling and provide

the first preclinical evidence that AAV-mediated silencing of IRBIT could represent a therapeutic approach for preventing or treating cardiac hypertrophy and progression to heart failure. Beyond revealing IRBIT's pathological role, this work highlights the translational potential of AAV-based strategies to correct deleterious molecular pathways in cardiovascular disease.

Gastrointestinal Physiology Symposium

“Novel Mechanisms in Liver Disease Pathogenesis”

INVOLVEMENT OF BONE MORPHOGENETIC PROTEINS IN THE PROGRESSION OF CHRONIC LIVER DISEASES

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The main feature in chronic liver diseases (CLD) is the hepatic fibrosis, which is triggered as a response against a chronic damage in the liver. The stage of hepatic fibrosis is the main risk factor to establish CLD prognosis. Metabolic dysfunction-associated steatotic liver disease (MASLD) is the main cause of CLD worldwide, being metabolic dysfunction-associated steatohepatitis (MASH) the most clinically relevant stage of the disease given its progressive profile. Currently, liver biopsy is still the gold standard to differentiate MASH and to accurately establish the fibrosis stage. However, it is considered an invasive method given its associated risks and complications. Thus, the search of new biomarkers for the diagnosis/prognosis of CLD is becoming more relevant in the latest years. Bone morphogenetic proteins (BMP) are growth factors belonging to the transforming growth factor β (TGF β) family, that have been involved in the regulation of different cellular processes and in the homeostasis of different organs and systems. Given that BMP proteins have already been proposed as biomarkers associated to different pathologies, this study aimed to establish their possible role in CLD progression. For this purpose, we have studied different experimental models of liver damage and cohorts of patients with liver disease. An experimental model of MASLD showed an increased hepatic expression of BMP2 in mice fed with high fat diet, and also in hepatocytes treated with palmitic acid. Similarly, in a cohort of biopsy-proven MASLD patients, an increased hepatic expression and serum concentration of BMP2 was observed when comparing to individuals with normal liver. Combining BMP2 circulating concentration with variables clinically relevant for MASLD, an algorithm named *Screening Algorithm for NASH* was developed, which showed high efficacy to discriminate MASH. On the other hand, different experimental models of fibrosis were generated, and an increased hepatic

expression of BMP8A was observed parallel to the fibrosis stage. Similarly, in a cohort of biopsy-proven MASH patients, an increased concentration of BMP8A in serum was observed in those MASH patients who presented advanced fibrosis (F3-F4). In fact, an algorithm based on BMP8A serum levels, named *BMP8A Fibrosis Score* (BFS), was developed, which allowed to predict efficiently advanced fibrosis in patients with MASH. Furthermore, BFS was validated in a larger patient cohort, showing higher diagnostic precision than other predictive scores, eliminating indeterminate results and improving risk stratification. Moreover, BMP8A is expressed and secreted by hepatocytes and hepatic stellate cells treated with TGF β , relating this BMP with fibrosis progression. In conclusion, these findings demonstrate the potential role of BMP proteins as biomarkers for the diagnosis and/or prognosis of CLD, and their contribution to the progression of liver diseases.

ROLE OF ACYL-COA BINDING PROTEIN IN FATTY LIVER DISEASE PROGRESSION: AN EMERGING THERAPEUTIC TARGET

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Metabolic dysfunction-associated fatty liver disease (MAFLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is the most prevalent chronic liver disorder worldwide and a major risk factor for fibrosis, cirrhosis, and hepatocellular carcinoma. Although lifestyle modifications remain the cornerstone of therapy, effective pharmacological options are still lacking. Acyl-CoA binding protein (ACBP), also referred to as diazepam binding inhibitor (DBI), has recently emerged as a novel regulator of hepatic metabolism and autophagy, with potential implications for disease progression.

ACBP/DBI expression is exacerbated in several conditions, including liver pathologies. We recently demonstrated that circulating ACBP/DBI is elevated in patients with metabolic steatohepatitis and fibrosis, correlating with histological severity and clinical scores such as NAFLD activity score and FIB-4, independently of age or body mass index. In preclinical studies, neutralization of extracellular ACBP/DBI with monoclonal antibodies (α -ACBP/DBI) exerted both prophylactic and therapeutic effects, protecting against the onset of steatosis and inflammation as well as reversing established liver injury. Specifically, α -ACBP/DBI treatment was effective in multiple mouse models, including methionine-choline deficient diet, western diet, ethanol diet and CCl₄-induced fibrosis. These beneficial effects were linked to restoration of autophagic flux, underscoring the role of ACBP/DBI as an extracellular “checkpoint” that restricts hepatoprotective autophagy.

Altogether, these findings identify ACBP/DBI as a central modulator of liver disease progression. Elevated extracellular ACBP/DBI may serve not only as a non-invasive biomarker but also as a therapeutic target. Its neutralization provides both prophylactic benefits, by preventing early metabolic imbalance, and therapeutic efficacy, by reducing steatosis, inflammation, and fibrosis in advanced stages. Thus, ACBP/DBI represents a promising target for biomarker discovery and monoclonal antibody-based interventions.

LIVER SINUSOIDAL ENDOTHELIAL CELLS DYSFUNCTION IN THE PROGRESSION OF MASLD

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Liver sinusoidal endothelial cells (LSECs) play a vital role in maintaining hepatic homeostasis. Due to their lack of a basement membrane and the presence of small pores organized into sieve plates called fenestrae, LSECs form a highly permeable barrier that enables bidirectional exchange of substrates between hepatocytes and the blood. In addition to their potent endocytic capacity mediated by specialized receptors, LSECs exhibit vasodilatory, anti-inflammatory, anti-thrombotic, and anti-fibrotic phenotypes, which are essential for regulating intrahepatic vascular tone, immune cell interactions, and preserving the liver's microenvironment. In disease condition, LSEC function and phenotype change. LSEC dysfunction, i.e. loss of vasodilatory capacity, has been implicated in the progression of chronic liver diseases. When exposed to pathological stimuli, LSECs undergo a process called capillarization, characterised by a reduction in the number and size of fenestrae, endothelial thickening and basement membrane formation. In addition, LSECs lose their protective properties, acquiring a pro-inflammatory and pro-fibrotic status interacting with hepatic stellate cells.

More specifically, in metabolic dysfunction-associated steatotic liver disease (MASLD), characterised by the accumulation of triglycerides within hepatocytes, LSECs dysfunction promotes the development of steatosis and drives the progression of MASLD to metabolic dysfunction-associated steatohepatitis (MASH). Using mouse models, several underlying mechanisms have been identified showing how excessive lipid exposure or the endothelial-specific loss of vascular modulators, such as semaphorin 3A, modulate LSECs fenestrae and sinusoidal permeability, promoting lipid retention and hepatic steatosis. Recent human studies

have shown that LSECs capillarisation occurs prior to the onset of MASH and correlates with disease severity and the extent of liver fibrosis. Importantly, treatment with the pan-PPAR (peroxisome proliferator-activated receptor) agonist Lanifibranor improved LSECs dysfunction and capillarization in both MASH patients and in rat preclinical models of MASLD and MASH.

On a mechanical point of view, LSECs autophagy impairment has been described as a mechanism involved in MASH progression at both the early and advanced stages. Liver biopsies from MASH patients revealed a reduced number of autophagic vacuoles in LSECs, indicating defective autophagy. Consistent with this, *in vivo* studies using mice deficient in endothelial autophagy demonstrated increased liver inflammation, liver apoptosis and enhanced fibrosis when exposed to a high-fat diet and chronic CCl₄ treatment.

Collectively, LSEC dysfunction plays a role in the development and progression of MASLD to MASH, representing a promising therapeutic avenue for restoring their endothelial function and mitigating liver disease progression.

Extracellular Vesicles Symposium

“Extracellular Vesicles in Physiological and Pathological Conditions”

THERAPEUTIC POTENTIAL OF NEURAL STEM CELL-DERIVED EXTRACELLULAR VESICLES IN NEURODEGENERATION

Mercyleidi Díaz Reyes¹ and Claudia Banchio¹

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by both motor and non-motor symptoms, caused by the degeneration and loss of dopaminergic neurons in the *substantia nigra*. Current therapies are limited to symptom management, unable to prevent neuronal loss or halt the progression of the disease. A significant limitation to more effective treatments is the difficulty of crossing the blood-brain barrier (BBB). Extracellular vesicles (EVs) communication plays a crucial role in several physiological processes within the nervous system. Given the rising prevalence of PD, the need for therapies that prevent neuronal death and promote cell survival is urgent. This study explores the potential of neural stem cell-derived small extracellular vesicles (NSC-EVs) using *in vitro* and *in vivo* models of PD. Our findings demonstrate that EVs purified from primary cultures of NSCs, significantly enhance the survival of dopaminergic neurons by reducing apoptosis and showing strong neuroprotective effects. Notably, the natural extracellular vesicles used in this study are enriched with Catalase, a potent scavenger protein with antioxidant properties. This enrichment further strengthens their

neuroprotective capacity, enabling them to mitigate oxidative stress and protect vulnerable neurons. The use of such naturally enriched extracellular vesicles represents a promising approach for developing innovative therapies to effectively combat Parkinson's disease.

MICRORNAS CONTAINED IN EXTRACELLULAR VESICLES: FROM POST-TRANSCRIPTIONAL MODULATORS TO DIAGNOSTIC BIOMARKERS OF HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer, often diagnosed at advanced stages due to insufficient early screening and monitoring. microRNAs (miRNAs) are key regulators of gene expression and potential biomarkers for cancer diagnosis. Our study investigated the diagnostic potential of miRNAs in Extracellular Vesicles (EVs) from HCC. miRNA expression in EVs was analyzed using HCC cell lines, circulating EVs from a Diethylnitrosamine (DEN)-induced liver tumor rat model, and plasma samples from HCC patients. Receiver Operating Characteristics (ROCs) were applied to evaluate the diagnostic accuracy of circulating EV miRNAs in patients. Five miRNAs (miR-183-5p, miR-19a-3p, miR-148b-3p, miR-34a-5p, and miR-215-5p) were consistently up-regulated in EVs across in vitro and in vivo HCC models. These miRNAs showed statistically significant differences in HCC patients stratified by TNM staging and Edmondson–Steiner grading compared to healthy controls. They also differentiated HCC patients with various etiologies from the control group and distinguished HCC patients, with or without liver cirrhosis, from cirrhotic and healthy individuals. Individually and as a panel, they demonstrated high sensitivity, specificity, and accuracy in identifying HCC patients. Their consistent upregulation across models and clinical samples highlights their robustness as biomarkers for HCC diagnosis, offering the potential for early disease management and prognosis.

TOTAL AND EXTRACELLULAR VESICLE cAMP CONTENTS IN URINE ARE ASSOCIATED WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) PROGRESSION

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic renal disorder, characterized by the progressive development of multiple renal cysts that ultimately lead to a decline in glomerular filtration rate and progression to end-stage renal disease. Intracellular cyclic adenosine monophosphate (cAMP) plays a pivotal role in cyst initiation and expansion, and represents a well-established therapeutic target. Inhibition of the vasopressin V₂ receptor by tolvaptan has been shown to reduce cAMP signaling and slow disease progression. However, direct measurement of renal intracellular cAMP is not feasible in clinical practice, making urinary cAMP a valuable and accessible surrogate biomarker.

Extracellular vesicles (EVs) have emerged as key mediators of intercellular communication, transporting diverse molecular cargo. Despite their potential role in ADPKD pathogenesis, the relevance of cAMP-EVs to disease progression remains poorly understood. Moreover, the relationship between cAMP-EVs, osmoregulation, and the natural course of ADPKD has not been previously addressed.

This lecture is focused on showing the contribution of both urinary total and EVs-associated cAMP to disease progression and water balance in ADPKD. Our findings demonstrate that total urinary cAMP correlates with osmolar clearance and established markers of disease progression, while cAMP-EVs appears to reflect underlying structural disturbances in the kidney. These results support the potential of urinary cAMP, both free and vesicle-content, as a biomarker of disease severity and progression. Furthermore, its relationship with hydration status suggests a possible role in assessing therapeutic response to tolvaptan.

Challenges in the isolation of EVs, such as low yield, contamination, and time-consuming protocols, have prompted the development of a novel method known as ExoExpress-U. This approach is currently being advanced by a nationwide multidisciplinary team of researchers to enable efficient urinary exosome capture. By utilizing magnetite particles conjugated with an anti-CD63 aptamer, ExoExpress-U could facilitate rapid and selective exosome isolation, minimize contamination, and enhance sample purity. This platform presents significant potential for biomarker discovery and translational research.

Altogether, our results highlight the diagnostic and therapeutic potential of urinary cAMP measurements in ADPKD and propose a new strategy for non-invasive monitoring of disease progression and treatment efficacy.

Oncology Symposium

“Inside the Cancer Cell: Pathway Disruption, Nuclear Armor, and Sensory Loss.”

**FROM COMPUTER TO THE LAB: RAC1 PATHWAY IN 5-FU-RESISTANT COLORECTAL
CANCER**

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Colorectal cancer (CRC) ranks as the third most frequently diagnosed malignancy worldwide and is a major contributor to cancer-related mortality. The chemotherapeutic agent 5-fluorouracil (5-FU), administered alone or in combination, represents a cornerstone of CRC treatment; however, nearly half of patients develop resistance to treatment. To explore the biological mechanisms underlying recurrence following 5-FU therapy, we performed an integrative study combining computational, cellular, and animal models. Using public gene expression datasets from patients, we identified differentially expressed genes between recurrent and non-recurrent phenotypes after 5-FU monotherapy (FDR ≤ 0.05 , $|\log_{2}FC| > 1$). These genes were subsequently subjected to pathway enrichment ($p \leq 0.05$), gene set enrichment (FDR-P ≤ 0.05 , $|NES| > 2$), and transcription factor motif analysis (FDR-P < 0.001 , $|NES| > 3$). We then constructed a combined gene expression matrix integrating patients treated with 5-FU monotherapy, FOLFOX, and FOLFIRI regimens, and applied feature selection coupled with machine learning to highlight predictive markers of recurrence. The analyses consistently pointed to the Rac1 pathway as a central mediator of resistance. Biochemical pull-down assays confirmed elevated Rac1 activity in resistant versus sensitive CRC cells. Leveraging drug repositioning resources, we identified compounds capable of restoring sensitivity to 5-FU ($p < 0.05$). Among them, the novel Rac1 inhibitor 1A-116 significantly decreased cell viability ($p < 0.05$), resensitized resistant cells to 5-FU, and reversed epithelial marker alterations and morphological changes associated with resistance, reinstating a phenotype comparable to controls. Finally, *in vivo* experiments demonstrated that 1A-116 effectively reduced tumor progression and metastatic spread. Altogether, our findings underscore Rac1 inhibition as a promising therapeutic strategy for CRC patients who develop resistance to 5-FU-based therapies.

NUCLEAR ENVELOPE IN PANCREATIC CANCER: THE ARMOR PROTECTING KRAS ONCOGENIC FUNCTION

Martin E. Fernandez-Zapico, Kayla C. LaRue-Nolan, Luciana L. Almada, Ashley N. Sigafos, Tara L. Hogenson, Luis F. Flores, David R. Pease, Laura Hilario Garcia, Maria F. Rodriguez-Quevedo

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Pancreatic ductal adenocarcinoma (PDAC), the most common histological subtype of pancreatic cancer, is a devastating disease predicted to be the second leading cause of cancer deaths by 2040. Despite extensive molecular characterization, therapeutic targeting of this disease remains largely unsuccessful. Thus, an increased understanding of the mechanisms driving malignant features of PDAC is critical for the development of new therapeutic strategies. Of these, cancer cell survival is a central malignant event in PDAC contributing to disease initiation, progression, and response

to therapy. To date, the majority of cell survival mechanisms associated with PDAC biology has highlighted the role of cytoplasmic and extracellular factors, making the role of nuclear elements understudied. Our preliminary data has demonstrated evidence of a novel mechanism regulating nuclear envelope stability, a key process contributing to cell survival. Work from our lab has shown that oncogenic KRAS, the major genetic driver of PDAC and present in ~40% of cases, is sufficient to reduce the incidence of spontaneous nuclear envelope rupture, a measure of nuclear envelope stability. Using laser-induced nuclear envelope rupture revealed the presence of oncogenic KRAS decreases the extent of ruptures, suggesting an axis downstream of oncogenic KRAS is key for regulation of nuclear stability. Analysis of nuclear lamins, key intermediate filaments providing structure to the nuclear envelope, revealed only A-type lamins, not B-type, are associated with overall PDAC survival and enrich at the nuclear periphery downstream of oncogenic KRAS. Furthermore, BioID protein-protein interaction assays to examine the oncogenic KRAS-induced effects on nuclear repair proteins identified an increase in interaction between Lamin A and Emerin. Knock-down of either protein results in increases in nuclear envelope spontaneous ruptures. Together our findings provide a novel oncogenic KRAS-Lamin A-Emerin axis controlling the nuclear stability in PDAC, which can serve as a new therapeutic avenue to improve PDAC treatment outcomes.

DECODING THE PRIMARY CILIUM: A KEY PLAYER IN CHOLANGIOCARCINOMA PATHOGENESIS

Sergio Gradilone.

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Primary cilia are solitary, microtubule-based organelles that function as cellular “antennae,” integrating mechanical and chemical cues to coordinate signaling pathways that regulate cell proliferation, differentiation, polarity, and repair. Once considered vestigial, primary cilia are now recognized as crucial sensory hubs across many tissues, including the liver. In cholangiocytes, the epithelial cells lining the intrahepatic bile ducts, cilia play essential roles in maintaining tissue

homeostasis and disruption or loss of these structures has emerged as a key pathogenic event in hepatobiliary disorders.

Our work has demonstrated that primary cilia loss contributes to the progression of polycystic liver disease (PLD) and cholangiocarcinoma (CCA), a highly lethal bile duct cancer. Mechanistically, deciliation alters the localization and activity of growth factor receptors, including the epidermal growth factor receptor (EGFR), resulting in aberrant signaling that drives uncontrolled proliferation and survival. In addition, ciliary loss reprograms cellular metabolism, enhancing glucose uptake and glycolytic flux while also engaging mitochondrial pathways to sustain energy demands. These metabolic adaptations not only fuel tumor growth but also create vulnerabilities that may be exploited therapeutically. Furthermore, recent studies show that the absence of cilia affects DNA damage responses and repair efficiency, linking ciliary dysfunction to genomic instability and disease progression.

Ongoing work is uncovering the molecular drivers of ciliary disassembly. Aberrant regulation of deacetylases such as HDAC6 and SIRT1 promotes ciliary resorption, thereby sustaining a deciliated state that favors disease. Importantly, pharmacological or genetic interventions targeting these pathways can restore cilia or mimic their signaling output, supporting the emerging concept of “ciliotherapy” as a strategy to prevent or reverse disease. By targeting ciliary resorption or harnessing cilia-dependent signaling, new avenues for therapeutic intervention in PLD and CCA are being defined.

Cholangiocyte primary cilia thus emerge as central regulators of liver health and disease, orchestrating signaling, metabolism, and DNA repair. These insights not only advance fundamental understanding of ciliary biology but also open new opportunities for therapeutic development.

Endocrinology Symposium

"The Skeletal Muscle: a new endocrine organ?"

RESTORATION OF MOTOR FUNCTION AFTER RECOMBINANT NETRIN-1 ADMINISTRATION IN A SEVERE CHRONIC SPINAL CORD INJURY MODEL

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Traumatic spinal cord injury (SCI), which is usually caused by contusion or compression, is a leading cause of permanent disability worldwide. In severe cases, the condition becomes chronic with irreversible loss of motor and physiological functions below the site of the injury. At this stage, the formation of a glial scar interrupts communication between the central nervous system (CNS)

and the periphery, and the CNS itself lacks sufficient regenerative capacity. These processes result in functional deficits and limit recovery. Current treatments are limited to rehabilitation, which only provides partial benefits depending on the severity of the lesion and the preservation of tissue. Therefore, there is an urgent need for new strategies that can promote neuronal repair and restore function in chronic SCI.

Netrin-1, a developmental guidance molecule critical for corticospinal tract formation, emerges as a potential therapy. In previous work, we demonstrated in an acute transection model that Netrin-1 stimulates axonal regrowth, reconnection, and recovery of locomotor function. Based on this evidence, we hypothesized that Netrin-1 could also act as a neuroreparative molecule in the chronic stage, where barriers to regeneration are more complex.

To evaluate this, we used a rat model of high-severity chronic SCI at thoracic levels Th10–Th11. Administration of Netrin-1 directly into the lesion site induced significant recovery of hindlimb motor activity. Treated animals regained extensive movement of hip, knee, and ankle joints, including complete flexion and extension, previously lost due to injury. In addition, they showed partial restoration of functional tasks such as climbing and grasping.

Histological and cellular analyses supported these functional improvements. Netrin-1 promoted regrowth, sprouting, and remyelination of corticospinal tract axons, reconnection of extrapyramidal fibers, regrowth of serotonergic and dopaminergic pathways, prevention of transsynaptic degeneration of motoneurons, and protection of both sensory and motor fibers.

In conclusion, this study demonstrates that Netrin-1 maintains reparative activity even in chronic SCI, enhancing axonal repair and functional recovery. These findings expand its therapeutic potential and support consideration of Netrin-1 as a candidate for translational approaches and future clinical trials in patients with severe chronic spinal cord injury.

ROLE OF MUSCLE IN CARDIOPULMONARY AND METABOLIC REHABILITATION

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Skeletal muscle occupies a central role in cardiopulmonary and metabolic rehabilitation. It is not only a structure compromised by systemic disease but also a decisive determinant of functional recovery. In patients with chronic heart failure, ischemic heart disease, chronic obstructive pulmonary disease, pulmonary hypertension, interstitial lung diseases, obesity, and type 2 diabetes, skeletal muscle undergoes profound alterations such as mitochondrial dysfunction, reduced oxidative capacity, and fiber type shifts. These adaptations result in early fatigability,

diminished exercise tolerance, and progressive inactivity, generating a self-perpetuating cycle of deconditioning that aggravates symptoms and worsens prognosis.

Modern rehabilitation considers muscle not merely as a passive target but as an active therapeutic agent. Exercise training induces favorable structural and metabolic adaptations. Aerobic programs improve ventilatory efficiency, peak oxygen consumption, and cardiovascular reserve. Resistance training enhances strength, peripheral circulation, and muscular endurance. Inspiratory muscle training contributes to reduced dyspnea and better ventilatory mechanics. Interval protocols, adapted to patient capacity, stimulate both central and peripheral pathways, producing superior outcomes compared with traditional continuous regimens.

The benefits extend beyond performance. Muscle-directed interventions improve insulin sensitivity, lipid metabolism, and systemic inflammatory profiles, contributing to metabolic balance and reduced cardiovascular risk. These effects translate clinically into fewer hospitalizations, reduced morbidity, improved autonomy, and a measurable increase in quality of life.

Strict monitoring is a cornerstone of safe and effective programs. Initial cardiopulmonary exercise testing establishes thresholds and individualizes prescriptions. Continuous electrocardiographic and oxygen saturation surveillance during training allows early detection of arrhythmias or desaturation. Regular metabolic and functional assessments ensure that adaptations are tracked and goals are adjusted.

In conclusion, skeletal muscle stands as a pivotal element in cardiopulmonary and metabolic rehabilitation. Its dysfunction amplifies disease burden, while its capacity for adaptation underlies the therapeutic potential of structured exercise. By integrating muscle physiology into rehabilitation protocols, clinicians shift from an organ-centered paradigm to a systemic approach where restoring muscular health is synonymous with restoring overall function. This perspective consolidates the role of muscle as both a vulnerable target and a powerful ally in improving outcomes for patients with chronic disease.

SKELETAL MUSCLE REVISITED: A FOCUS ON ITS CARDIOMETABOLIC FUNCTION.

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Skeletal muscle is increasingly recognized not just as a mechanical tissue but also as an endocrine organ that secretes bioactive peptides and proteins—myokines—in response to contraction. The concept of muscle as an endocrine organ, developed in recent decades, partially answers the issue of how the crosstalk between skeletal muscle and different organs and tissues occurs. Systemic responses to exercise (particularly during muscle contraction) exert autocrine

and paracrine effects on skeletal muscle cells, and endocrine effects in distal organs such as the heart, kidney, brain, adipose tissue, and liver.

In the cardiovascular system, myokines contribute to endothelial health, blood pressure regulation, anti-inflammatory balance, cardiac remodeling, and modulation of risk factors. The link between myokines and cardiovascular disease is essential for developing comprehensive treatments and new biomarkers. Several myokines, including myostatin, irisin, brain-derived neurotrophic factor, mitsugumin 53, meteorin-like, apelin, follistatinlike 1, decorin, and myogenin, participate in regulating the pathogenesis of cardiovascular diseases (CVD). Measuring these myokines in peripheral blood offers novel perspectives on CVD advancements and enables clinicians to stratify patients by their risk.

Moreover, myokines such as myostatin, irisin, brain derived neurotrophic factor, mitsugumin 53, meteorin-like, and apelin the potential roles in various CVD, including myocardial infarction, heart failure, atherosclerosis, and hypertension. Furthermore, this review highlights the emerging role of myokines in CVD and the challenges remaining in translating these discoveries into novel clinical biomarkers for CVD.

CONGRESS ABSTRACTS

Cardiovascular Physiology and Hypertension

CV 01

CALCIUM-DEPENDENT MORPHOLOGICAL ALTERATIONS IN ISOTONIC ENVIRONMENTS IN PATHOLOGICAL CARDIAC HYPERTROPHY: A NEW ROLE FOR CALCIUM?

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INTRODUCTION Calcium (Ca^{2+}) is a key divalent ion in cardiac contractile mechanics. Proper regulation of Ca^{2+} handling is essential for tissue function; however, cell volume—and thus morphology—also plays a critical role in contractile performance. Cell volume regulation depends mainly on the flow of inorganic ions such as sodium (Na^+), potassium (K^+), and chloride (Cl^-) across the sarcolemma. The contribution of Ca^{2+} flux to this process, however, remains under investigation. Moreover, changes in cardiac cell morphology under isotonic conditions are not yet fully understood and are of particular interest in this study.

OBJECTIVES To investigate the role of extracellular Ca^{2+} increase in the morphology and functionality of cardiomyocytes from normotrophic and hypertrophic hearts under isotonic conditions.

METHODS Transverse aortic constriction (TAC -T, N=15-) surgery was performed in 10-week-old male C57Bl/6 mice (SHAM -S, N=16- as control). Additionally, H9C2 cell cultures (n=6 wells per condition), with or without exposure to Angiotensin II (AngII, 1 μ M), were employed. Cell area was quantified, and epifluorescence/confocal microscopy and patch-clamp techniques, among others, were used. t-Test (t) and one-way Anova (A) test were used. A $p < 0.05$ was considered statistically significant (*).

RESULTS TAC successfully induced cardiac hypertrophy, confirmed by increased atrial natriuretic peptide (ANP) expression (20-fold increase, t *) and higher heart weight/body weight ratios (25%-fold, t *) . Compared with SHAM, TAC cardiomyocytes exhibited significant increases in cell area, length, and width when extracellular Ca^{2+} concentration ($[Ca^{2+}]$) was elevated (around 15%-fold of each parameter, A *). Furthermore, H9C2 cells exposed to AngII, but not the control, displayed a significant Ca^{2+} -dependent increase in cell area (around 15%-fold at 2 mM from 0 mM $[Ca^{2+}]$, A *).

CONCLUSION Elevated extracellular Ca^{2+} under isotonic conditions induces morphological changes exclusively in cardiomyocytes with maladaptive hypertrophy. This phenomenon warrants further investigation to clarify its pathophysiological implications.

CV 02

IS ZINC SUPPLEMENTATION DURING GROWTH BENEFICIAL TO DIMINISH THE CARDIOVASCULAR AND METABOLIC ALTERATIONS OF METABOLIC SYNDROME?

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Introduction: Zinc supplementation may reduce metabolic and cardiovascular damage associated with metabolic syndrome, given zinc's antioxidant and anti-inflammatory properties.

Objective: To evaluate the effects of zinc supplementation on systolic blood pressure (SBP), intermediate metabolism and retroperitoneal adipose tissue (RPAT) in male Wistar rats fed a high-fat and fructose diet (HFFD) during post-weaning growth.

Methods: Male Wistar rats were fed from 21 to 81 days of life: control diet (CC, Zinc 30 ppm), zinc supplemented diet (CZ: Zinc 190 ppm), high-fat diet (CFF; 60% pork fat calories, 30 ppm Zinc) and 10% fructose in drinking water, high-fat diet supplemented with zinc (ZFF; 60% fat calories, 190 ppm Zinc) and 10% fructose in drinking water. SBP, oral glucose tolerance test (OGTT), lipid profile, and adipokine ARNm expression, morphological changes and oxidative state in RPAT were evaluated at 81 days. Statistics: 2-way ANOVA, post-hoc test: Bonferroni: * $p < 0.05$ vs CC; * $p < 0.05$ vs CFF; & $p < 0.05$ vs CZ; $n = 9$ per group.

Results: ZFF rats showed reduced SBP, insulin resistance, triglyceridemia, and cholesterol compared to CFF. ZFF rats showed reduced adipocyte hypertrophy (CC: 5258 ± 312 ; CZ: $3070 \pm 208^*$; CFF: $7203 \pm 454^*$; ZFF: $5368 \pm 549^{+ \&}$ μm^2) and media layer area/light area of vessels (CC: 2.2 ± 0.4 ; CZ: 3.1 ± 0.5 ; CFF: $4.8 \pm 0.4^{**}$; ZFF: $2.7 \pm 0.3^{++}$), lower thiobarbituric acid reactive species concentration (CC: 0.21 ± 0.01 ; CZ: $0.09 \pm 0.02^*$; CFF: $0.38 \pm 0.03^*$; ZFF: $0.23 \pm 0.04^{+ \&}$ nmol/mg protein), higher antioxidant superoxide dismutase activity (CC: 5.8 ± 0.3 ; CZ: $12 \pm 2^*$; CFF: 6.4 ± 0.7 ; ZFF: $11 \pm 2^+$ uSOD/mg protein) and NRF2 mRNA expression (CC: 2.3 ± 0.4 ; CZ: $8.9 \pm 1.1^*$; CFF: 1.3 ± 0.2 ; ZFF: $4.6 \pm 0.7^{+ \&}$).

Conclusion: Zinc supplementation during growth reduces cardiovascular and metabolic damage induced by HFFD. In CZ rats, zinc improves insulin resistance, glycemia, cholesterol levels, oxidative stress in RPAT.

CV 03

T-TUBULE REMODELING AND EARLY CALCIUM ALTERNANS PERSIST DESPITE BLOOD PRESSURE LOWERING IN SPONTANEOUSLY HYPERTENSIVE RAT MYOCYTES

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Introduction: In spontaneously hypertensive rats (SHR), chronic hypertension promotes left ventricular hypertrophy, T-Tubule (TT) disorganization and increased susceptibility to Ca alternans, beat-to-beat oscillations in action potential duration, Ca transients, and contraction strength that often precede severe arrhythmias. We previously showed that the phosphodiesterase-5 inhibitor sildenafil, with vasodilatory effects, prevents premature Ca alternans and TT remodeling. However, it remains unclear whether these protective effects result from hemodynamic changes or direct actions on cardiomyocytes. To assess the vascular contribution, we tested an alternative blood pressure-lowering strategy using diuretics.

Objective: To determine whether the early onset of Ca alternans and TT remodeling in SHR is prevented by reducing cardiac loading conditions.

Methods: SHR rats were treated with a combination of Hydrochlorothiazide (diuretic, 9mg/kg/day) and Hidralazine (vasodilator, 44 mg/kg/day), referred to as HH, from 3 to 6 months of age in drinking water. Blood pressure was measured before and after treatment. Ca dynamics and frequency-induced alternans, were assessed in isolated myocytes loaded with a Ca-fluorescent indicator. TT organization was analyzed by confocal microscopy using Di-8-ANEPPs.

Results: HH treatment significantly reduced systolic blood pressure (from 189 ± 8 to 117 ± 5 mmHg, $n=5$). Myocytes from HH-treated animals did not show changes in the threshold frequency for the onset of alternans compared with control SHR group (SHR: 4.3 ± 1.1 , HH: 4.9 ± 0.5 Hz; $n=20/5$, cells/hearts per group). No differences were observed in the refractoriness of sarcoplasmic reticulum Ca release (time to 50% recovery of Ca transient: SHR: 313 ± 2 , HH: 306 ± 3 ms) or in TT organization measured by TT power (SHR: 54.8 ± 0.5 , HH: 1 ± 0.7 au).

Conclusions: Although HH treatment effectively reduced blood pressure, it failed to prevent TT disorganization or the premature appearance of alternans in SHR myocytes. These findings indicate that factors beyond hemodynamic changes contribute to TT remodeling and increased vulnerability of arrhythmogenic cardiac alternans in hypertrophic hearts.

CV 04

CALCIUM-CALMODULIN-DEPENDENT PROTEIN KINASE PARTICIPATION IN THE MOLECULAR PATHWAYS OF THE SLOW FORCE RESPONSE TO MYOCARDIAL STRETCH

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Introduction. Myocardial stretch causes a biphasic contractile response: The Frank-Starling mechanism, followed by a slower increase in force called slow force response (SFR) or Anrep effect. The mechanisms underlying the SFR are not completely understood. We previously demonstrated that these mechanisms comprise a series of steps culminating in the activation of the Na^+/H^+ exchanger, resulting in elevated intracellular Na^+ level and a subsequent increase in intracellular Ca^{2+} concentration via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Recent experiments proposed that the SFR depends on calcium-calmodulin-dependent protein kinase (CaMKII) activation through nitrosylation, suggesting that CaMKII-dependent phosphorylation of ryanodine receptors (RyR2)

underlies the increased sarcoplasmic reticulum Ca²⁺ transients observed with increased afterload. However, the role of RyR2 was not directly assessed.

Objective: This study was aimed to link the previously demonstrated cascade with CaMKII activity.

Methods. The experiments were performed in isometrically contracting (0.2 Hz) isolated papillary muscles from Wistar rats, cats, and Ser2814A mice (CaMKII phosphorylation site on RyR2 was mutated to alanine to prevent phosphorylation). After defining slack length the muscles were gradually stretched to their maximal length (L_{max}). The SFR was determined after an abrupt stretch from ~92 to 98 of L_{max}.

Results. The SFR was suppressed in papillary muscles from Wistar rats after CaMKII inhibition (1mM autocalmitide-2 related inhibitory peptide): (in % of initial rapid phase) 126±1 (n=5) control vs. 101±1 (n=5) AIP (p<0.05); and from Ser2814A mutant mice: 127±4 (n=4) control, vs. 106±2 (n=5) Ser2814A (p<0.05). Furthermore, the NOS inhibitor L-NAME (1 mM) canceled the SFR in cat papillary muscles: 122±1 (n=4) control, vs. 105±1 (n=4) L-NAME (p<0.05), without affecting the increase in intracellular Na⁺ that underlies the SFR: (Dmmol/L vs. pre-stretched control): 2.82±0.38 (n=4) control, vs. 2.98±0.19 (n=4) L-NAME.

Conclusions. The results confirm the role of CaMKII in the SFR demonstrating for the first time that CaMKII-dependent phosphorylation of RyR2 plays a critical role in the development of the SFR.

CV 05

EFFECT OF CB1 CANNABINOID RECEPTOR SILENCING IN THE CONTEXT OF CARDIAC PATHOLOGY

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Introduction: Chronic marijuana use induces structural, mechanical, and electrical cardiac dysfunctions. Most studies agree that these deleterious effects are derived from the action of tetrahydrocannabinol (THC) and/or cannabidiol (CBD) on CB1r, resulting in lower contractility and blood pressure.

Objectives: Based on the differences observed in the literature regarding the role of these receptors, we aimed to investigate the role of differential activation of receptors in models of cardiovascular pathologies and their respective controls, by using adeno-associated viral vectors to differentially silence CB1r (AAV9-shCB1r) or CB2r (AAV9-shCB2r).

Methods: We administered AAV9-shCB1r and its respective control virus, AAV9-shControl, via intracardiac injection into 4- to 5-day-old male Spontaneous hypertensive rats (SHR) and performed a series of studies to assess cardiac hypertrophy, blood pressure, and ischemia/reperfusion response. Data are expressed as mean \pm standard error of the mean and were compared using Student's t-test or two-way ANOVA as corresponding.

Results: Four months after injection, we found that rats injected with AAV9-shCB1 showed a smaller infarct size after staining with triphenyltetrazolium chloride (TTC) salts, and also showed a higher developed pressure. In addition, we found an increase in left ventricular mass index (LVMI), heart weigh and left ventricle, all of them normalized for tibia length. Finally, we observed no change in rat blood pressure after silencing CB1.

Conclusion: Based on the results obtained so far, we can conclude that the silencing of CB1 caused an increase in preexisting hypertrophy in the animal model used. However, the mechanism by which this occurs still needs to be clarified, as well as whether this represents an exacerbation of pathological hypertrophy or the development of physiological hypertrophy. On the other hand, we conclude that CB1 silencing decreases infarct size and improves systolic function after ischemia-reperfusion injury.

CV 06

CHRONIC EFFECT OF APOA-I ON HYPERTENSION-INDUCED CARDIAC HYPERTROPHY

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Introduction: Sustained arterial hypertension promotes pathologic cardiac hypertrophy (PCH). High-density lipoprotein (HDL) and its main apolipoprotein, ApoA-I, have been demonstrated to exert cardioprotective effects beyond their anti-atherogenic role, although the underlying mechanisms remain poorly understood.

Objective: To evaluate the impact of chronic ApoA-I administration on PCH in spontaneously hypertensive rats (SHR).

Methods: Male SHR (3–4 months old) received intraperitoneal injections of either phosphate-buffered saline (PBS, control) or ApoA-I (0.2 μ g/ml) every 48 hours for 28 days. We assessed: (1) plasma lipid profile (colorimetric assay), (2) mean arterial pressure (MAP, telemetry), (3) cardiac structure and function (echocardiography and histology), (4) myocardial compliance

(tension–length relationship), and (5) signaling pathways (immunoblot). Data are expressed as mean±SEM; comparisons were performed with Student's t-test (p<0.05).

Results: ApoA-I did not significantly modify HDL-cholesterol (55.6±3.1 vs 50.6±2.4 mg/dl), total cholesterol (89.1±1.8 vs 88.6±1.9 mg/dl), or MAP (143±0.6 vs 146±1.4 mmHg, p=0.42). However, ApoA-I reduced cardiac hypertrophy, as evidenced by decreased left ventricular mass index/tibia length (22.4±0.5 vs 28.3±1.2 mg/mm, p=0.0013) and posterior wall thickness (1.81±0.02 vs 2.13±0.06 mm, p=0.014). Cardiomyocyte area remained unchanged (408±26 vs 369±34 µm², p=0.38), while left ventricular weight/tibia length showed a nonsignificant trend toward reduction (27.3±0.7 vs 29.4±0.8 mg/mm, p=0.078). Importantly, ApoA-I improved systolic performance (fractional shortening: 57.8±1.0 vs 54.7±0.9%, p=0.05) and myocardial compliance (1.03±0.05 vs 1.72±0.23, p=0.02). Akt phosphorylation displayed a nonsignificant increase (121±11.7 vs 100±7.4%, p=0.15)

Conclusion: These preliminary findings support that chronic ApoA-I administration attenuates PCH and improves myocardial function in SHR, supporting a cardioprotective role independent of lipid modulation.

CV 07

EFFECTS OF AN *Eragrostis tef*-ENRICHED DIET ON CARDIOVASCULAR AND METABOLIC ALTERATIONS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Introduction: Cardiovascular diseases are the leading cause of death worldwide, and arterial hypertension (AH) is the main risk factor for their development. The spontaneously hypertensive rat (SHR) model is widely used to study AH and its associated metabolic comorbidities, such as increased oxidative stress and insulin resistance. Diet is a key modulator in these processes. Teff (*Eragrostis tef*), a pseudocereal rich in polyphenols and fiber, has shown antioxidant properties and potential metabolic benefits, although its impact on AH associated with insulin resistance has not been sufficiently explored.

Objectives: To evaluate the effect of a teff-enriched diet (50%) on cardiac function, oxidative stress, glucose metabolism, and blood pressure in SHR compared with a standard diet.

Methods: Male SHR were divided into two groups (n=6 each): standard diet (C) and teff-enriched diet (50%) (T) for 8 weeks. Blood pressure (BP) was recorded by plethysmography, and

echocardiography was performed to assess cardiac function. Intraperitoneal glucose tolerance was tested, and plasma antioxidant activity was quantified using the FRAP method. Data were analyzed using *t*-test and ANOVA ($p < 0.05$).

Results: Feeding efficiency was higher in group T compared with C (0.062 ± 0.003 vs 0.049 ± 0.003). Plasma antioxidant activity in T was significantly higher than in C (0.067 ± 0.006 vs 0.048 ± 0.003 ug/ul). Group T showed a significant reduction in systolic BP (191.8 ± 4.06 vs 166.5 ± 3.55 mmHg) and diastolic BP (163.8 ± 3.61 vs 132.3 ± 3.15 mmHg) ($p < 0.05$), as well as a significant improvement in cardiac function, as determined by fractional shortening. Glucose tolerance improved in T, as evidenced by lower area under the curve values.

Conclusion: Dietary supplementation with teff exerts beneficial effects in SHR by reducing BP, improving cardiac function, enhancing glucose homeostasis, and attenuating oxidative stress. These findings suggest that teff could represent a promising nutritional strategy for the management of AH and its metabolic complications.

CV 08

EFFECTS OF ISCHEMIC POSTCONDITIONING IN HEARTS SUBJECTED TO ISCHEMIA-REPERFUSION: INFLUENCE OF METABOLIC CONDITIONS

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Introduction: Ischemic postconditioning (IPostC) is a potent cardioprotective strategy which consists of brief cycles of reperfusion(R)-ischemia(I) after the ischemic period. This intervention has elicited particular interest due to its potential clinical application. However, its translation to clinical practice has not been successful yet.

Objectives: The aim of this study was to compare the effects of IPostC in rat hearts subjected to I-R in different metabolic conditions.

Methods: Female Sprague-Dawley rats (220-270g), fed ad libitum or fasted for 24h, were used. Hearts were isolated and perfused using the Langendorff method. After a 25min stabilization period, hearts were subjected to 25min I-60min R. IPostC consisted of 6 cycles of 10sec R-10sec I at the onset of R.

Contractile function was assessed using a latex balloon and pressure transducers. Infarct size was determined by the TTC method. Tissue ATP content was assessed by luciferin/luciferase

bioluminescence assay. In isolated mitochondria, rate of ATP synthesis, mitochondrial membrane potential ($\Delta\Psi_m$) and respiratory complexes I-III, II-III and IV activities were evaluated. ANOVA, n=6/group.

Results: In hearts from fed rats, IPostC improved contractile function (both systolic and diastolic), decreased infarct size and increased post-ischemic tissular levels of ATP ($p < 0,05$ vs. fed Control). In hearts from fasted rats, however, there were no significant differences between the IPostC and Control groups in regards to these parameters.

In hearts from fed rats, IPostC improved mitochondrial ATP synthesis rate and Complex I-III and II-III activity ($p < 0,05$ vs. fed Control). This effect was not observed in IPostC hearts from fasted rats. $\Delta\Psi_m$ was also preserved by IPostC in fed rat hearts, while IPostC hearts from fasted rats showed greater despolarization ($p < 0,01$ vs. fasted Control).

Conclusion: Results suggest that IPostC exerts cardioprotective effects in hearts from fed rats, while these beneficial effects are abolished in hearts from fasted rats. These findings highlight the need for further investigation on the influence of cellular metabolism in the implementation of cardioprotective strategies.

CV 09

EFFECT OF FASTING ON THE CARDIOPROTECTIVE ACTION OF STEVIOSIDE IN AN EX VIVO ISCHEMIA-REPERFUSION MODEL

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Introduction: Stevioside, a natural glycoside from *Stevia rebaudiana Bertoni*, has shown cardioprotective properties in ischemia-reperfusion (Is-Rs) models. Since fasting induces a metabolic shift toward fatty acid utilization, we investigated whether this condition modifies the effects of stevioside.

Objectives: To evaluate the impact of fasting on cardiac and mitochondrial responses to stevioside treatment in a Langedorff perfused Is-Rs model.

Methods: Female Sprague-Dawley rats were treated orally with stevioside (168 mg/kg/day, 15 days) and divided into fed (S) and 24 h-fasted (SF) groups, with corresponding controls (C, CF). Isolated hearts underwent 25 min global ischemia and 60 min reperfusion. Cardiac function (rate pressure product, RPP; $\pm dP/dT$, left ventricular end-diastolic pressure, LVEDP; infarct size) was assessed. Mitochondria were energized with pyruvate/malate or succinate to evaluate ATP

synthesis, respiratory complex activities, calcium retention capacity (CRC), and membrane potential ($\Delta\psi$). Echocardiography was also performed. ANOVA, n=6/group.

Results: No significant differences were observed in echocardiographic parameters. At 5 min reperfusion, stevioside improved RPP recovery in both fed and fasted groups ($p < 0.05$ vs C and CF), but this benefit was transient in SF and lost after 10 min. Fasting abolished differences previously seen in $+dP/dT$ and LVEDP between S and C. Stevioside reduced infarct size in fed ($p < 0.05$) but not in fasted rats. Fasting reduced pre-ischemic tissue ATP, but post-Is-Rs levels were similar among groups. Mitochondrial ATP synthesis and complex I–II activities were markedly reduced by fasting ($p < 0.001$ vs C and S), abolishing differences between stevioside and control. CRC significantly increased in pre-ischemic fasted groups ($p < 0.001$ vs C and S), with better SF preservation post-Is-Rs. $\Delta\psi$ showed substrate- and state-dependent changes; fasting eliminated the pre-ischemic differences between stevioside and controls.

Conclusion: Fasting markedly modifies mitochondrial metabolism and attenuates stevioside cardioprotection. These results highlight the importance of metabolic state in determining drug-induced cardioprotection.

Respiratory physiology

Resp 01

NEONATAL ETHANOL INTOXICATION DISRUPTS VENTILATORY AND METABOLIC ADAPTATIONS: STUDY AGAINST INTERMITTENT OR CONTINUOUS HYPOXIA

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Introduction: Acute ethanol (EtOH) intoxication depresses the hypoxic ventilatory response (HVR), which varies with the type of hypoxic stimulus. During continuous hypoxia (CH), ventilation initially increases but rapidly declines, even when hypoxia persists. In contrast, intermittent hypoxia (IH) elicits a more sustained HVR that reappears whenever O₂ levels drop again. Little is known about how acute EtOH effects modifies these responses during early postnatal life. Since serotonin (5HT) modulates HVR, and is also vulnerable to EtOH effects.

Objectives: How acute EtOH intoxication disrupts respiratory responses to 2-hypoxic challenges (CH vs IH).

Methods: Neonatal Wistar rats (n=57 pups, 7-8 pups/group) were used. On postnatal day 9, pups received 2.0 or 0.0 g/kg EtOH (i.g.) and were then subjected to normoxia, IH, or CH (O₂ 8%, 15 min), followed by recovery normoxia. Ventilatory parameters were measured in whole-body plethysmography, and brainstem and trunk blood were collected for 5HT quantification and metabolic assays. Statistical analyses were conducted through 3-way MANCOVAs, MANOVAs or ANOVAs; $\alpha < .05$.

Results: EtOH depressed HVR under both hypoxic protocols, though the expected response pattern was preserved ($p < .01$). Breathing depression, characteristic of the recovery normoxia (RN), was affected by EtOH only after IH, indicating a more adaptive ventilatory response against CH under intoxication (i.e. respiratory rates during the initial hypoxic period and RN were similarly to each other). Intoxicated pups elicited fewer apneas ($p = .01$) and lower 5HT levels than controls ($p = .01$), regardless of air condition. Both hypoxic challenges induced hypocapnia ($p < .01$), but EtOH leads to hypercapnia/acidosis ($p = .02$; $p < .01$). CH improved oxygenation (increasing PO₂ ($p = .03$) & oxyhemoglobin ($p = .03$)), but this effect was blunted in EtOH-pups.

Conclusions: Acute neonatal EtOH exposure compromises respiratory plasticity by impairing ventilatory and metabolic adaptations to hypoxia and reducing brainstem 5HT levels. CH appears less deleterious than IH during intoxication, yet both paradigms highlight the vulnerability of the ventilatory system during early development

Gastrointestinal physiology

Gast 01

FENOFIBRATE ATTENUATES OXIDATIVE STRESS GENERATION IN ESTROGEN - INDUCED CHOLESTASIS

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Introduction: Estrogens can trigger cholestasis in predisposed women during pregnancy or oral contraceptive use. 17 α -ethinylestradiol (EE) is used experimentally to reproduce this condition in rats. Oxidative stress (OS) is central to cholestasis pathogenesis, and activation of nuclear receptors such as PPAR- α and Nrf2 is a promising therapeutic strategy. PPAR- α , activated by fenofibrate (FF), exerts antioxidant effects partly through Nrf2 stimulation.

Objectives: To evaluate whether activation of PPAR α by FF attenuates OS in EE-induced cholestasis.

Methods: Male Wistar rats were randomly divided into the following groups: i) Control (C), ii) EE (5 mg/kg/day, i.d., 5 days), iii) FF (200 mg/kg/day, p.o., 7 days), and iv) EE+FF. To evaluate the hepatocellular redox status, the following parameters were assessed in liver tissue: (i) membrane lipid peroxidation, (ii) total oxidant and antioxidant levels, and (iii) activity of antioxidant enzymes, such as SOD, CAT, and GSH-Px.

Results:(*p<0.05 vs. control; #p<0.05 vs. EE). FF normalized SOD and GSH-Px hepatic activity levels, which had been increased (+58%, +68%, respectively) by EE. Hepatic CAT activity was also increased by EE (+41%*, C: 194.3 \pm 17.7; EE: 274.9 \pm 19.0) and partially normalized in the EE+FF group (233.2 \pm 16.1#). Lipid peroxidation, assessed by TBARS, was markedly increased by EE (+41%, C: 34,9 \pm 1,9; EE: 49,2 \pm 4,5), and was significantly reduced in EE+FF rats (27,6 \pm 4,1#). Total hepatic antioxidant capacity, which had been reduced by EE (-35%, C: 0.35 \pm 0.02 EE: 0.23 \pm 0.02), was restored by EE+FF (0.36 \pm 0.04#). Inversely, total oxidant levels were markedly increased in EE-treated rats (+151%, C: 1.23 \pm 0.20; EE: 3.08 \pm 0.64) and were significantly reduced in the EE+FF group (1.92 \pm 0.24#), approaching values observed in controls.

Conclusions: We conclude that FF reinforces the hepatic antioxidant defenses in EE-induced OS by reducing lipid peroxidation, restoring total antioxidant capacity, and lowering total oxidant levels. This protective effect is associated with the normalization of SOD, GSH-Px, and CAT activities, thereby enhancing liver's capacity to counteract oxidative damage.

Gast 02

DEVELOPMENT OF AN *EX VIVO* MODEL FOR THE STUDY OF THE ROLE OF BILIRUBIN GLUCURONIDE HOPPING IN LIVER PROTECTION AGAINST OXIDATIVE INJURY

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Introduction: Bilirubin (BR) bears antioxidant properties that protect the liver from oxidative stress-induced cholestatic injury. While the majority of glucuronidated BR is excreted into the bile canaliculus, a minor fraction is secreted back into the sinusoidal compartment, from where it re-enters neighboring hepatocytes. This process, known as hopping, may contribute to establish a more uniform protective BR gradient across the hepatic lobule.

Objective: To develop an *ex vivo* model of isolated perfused rat liver for the study of the role of BR glucuronide hopping in liver protection against oxidative injury induced by *tert*-butylhydroperoxide, *t*BuOOH.

Methods: To test the occurrence of hopping, we used indomethacin (Ind) at a concentration that simultaneously inhibits sinusoidal uptake and enhances canalicular excretion of BR glucuronides. Male Wistar rat livers were isolated and perfused *in situ* with Krebs buffer (pH 7.4; 37°C; 40 ml/min; 5/95 %v/v CO₂/O₂), and assigned to 4 experimental groups: Control (C, Krebs buffer), T (100 µM *t*BuOOH), BR/T (10.5 µM BR + 100 µM *t*BuOOH), and BR/Ind/T (400 µM Ind + 10.5 µM BR + 100 µM *t*BuOOH). Bile samples were collected every 5 min for 1 h for gravimetric assessment of bile flow. At the end of perfusion, livers were removed and stored for redox assays and histopathological evaluation.

Results: While *t*BuOOH markedly reduced bile flow [$p < 0.05$ vs C] and increased lipid peroxidation [T: 1.05 ± 0.71 vs C: 0.59 ± 0.18], BR pretreatment (BR/T group) partially attenuated these alterations [$p < 0.05$ vs T for bile flow; BR/T: 0.71 ± 0.25 , $p < 0.05$ vs T for TBARS]. Conversely, Ind suppressed the antioxidant protection afforded by BR [BR/Ind/T: 1.06 ± 0.42], while exerting no effect on bile flow [$p > 0.05$ vs C]. Histopathological analysis of liver samples showed necrosis and fibrosis, which were more noticeable in T than in the other experimental groups.

Conclusion: BR attenuated the deleterious effects of *t*BuOOH-induced oxidative injury, confirming its hepatoprotective role, while Ind abolished antioxidant protection conferred by BR under oxidative conditions, probably due to Ind-induced increased biliary excretion of glucuronidated BR.

Gast 03

AMPK activators metformin and alpha-lipoic acid reduce transdifferentiation and collagen deposition in hepatic-stellate derived cells.

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Introduction: Fibrosis is a hub condition in advanced liver disease that can progress to cirrhosis and hepatocellular carcinoma. The origin of collagen deposition in the extracellular matrix are hepatic stellate cells (HSCs) which activate and transdifferentiate into myofibroblasts in response to stress signals. The incidence of non-alcoholic fatty liver disease has risen, and preventive dietary and pharmacological approaches are proposed including treatment with metformin. However, clinical trials and studies on the cellular mechanisms of putative antifibrogenic effects of certain drugs are still needed.

Objectives: Herein, we investigated the effects of the AMPK activators, metformin and alpha-lipoic acid, alone and in combination on LX-2 cells (an HSC-derived cell line).

Methods: We used concentrations that, in our previous studies, showed anti-migratory effects on hepatic tumor cells linked to an AMPK-p53 axis. LX-2 cells were treated for 24 h with control medium (C), metformin (MET, 1 mM), alpha-lipoic acid (ALA, 0.5 mM), or both, in the presence or absence of the canonical HSC activator, TGF- β 1 (10 ng/ml). We then evaluated the levels of pAMPK, p53, α SMA, and COL1A1 by immunodetection. Collagen deposition was also analyzed by immunofluorescence microscopy.

Results: MET and ALA blocked the TGF- β 1-induced increase in COL1A1 levels, in parallel with a decrease in α SMA. COL1A1 levels in the ALA group were halved compared to the control group, and combination treatment showed a tendency of a more potent inhibition of fibrogenesis (mean values in TGF- β 1 groups C: 100; MET: 79*; ALA: 48*; MET-ALA: 38%*. *p< 0.05 vs. C). This was concomitant with AMPK activation, as pAMPK levels were significantly higher in the presence of both activators. However, p53 levels were maintained without significant variations.

Conclusion: Both MET and ALA strongly reduce the activation and collagen production of LX-2 cells. This effect is associated with AMPK activation. Further studies are needed to confirm AMPK signaling as a negative regulator of LX-2 cell transdifferentiation. Additionally, the enhanced antifibrotic effect observed is promising for considering combination therapies.

Gast 04

THE PREBIOTIC INULIN ATTENUATES ESTROGEN-INDUCED CHOLESTASIS IN A MURINE MODEL

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Introduction. Estrogen-induced cholestasis is a frequent pathology in susceptible women. In a rodent model of this condition, treatment with ethinyl estradiol (EE) reduces bile flow and bile salt excretion by altering hepatobiliary transporters. This treatment also increases inflammatory cytokines, which may contribute to cholestasis. Inulin (I), a prebiotic, can modulate gut microbiota and intestinal permeability, reducing bacterial product translocation (e.g., LPS) and inflammation.

Objectives. To evaluate the effect of inulin treatment in the EE-induced cholestasis model.

Methods. Male C57BL/6 mice were randomly assigned to four groups: (a) Control (vehicle); (b) EE (10 mg/kg/day, s.c., 5 days); (c) I (1g/kg/48h, p.o. 3 doses); or (d) EE+I. The common bile duct was catheterized via a trans duodenal surgery and bile was collected for 30 min. Plasma samples were used to measure alkaline phosphatase (ALP). Bile and liver samples were collected to measure bile salt excretion, protein levels of the hepatobiliary transporters BSEP and MRP3, and the transcription factor FXR.

Results. (Mean \pm SEM. n=3. * p<0.05 vs. Control. # p<0.05 vs. EE). Inulin treatment tended to normalize the decrease in bile flow (μ l/min/g liver) (Control: 1.4 \pm 0.1; EE: 1.1 \pm 0.1; EE+I: 1.6 \pm 0.2),

and in bile salt excretion rate (nmol/min/g liver) (Control: 48±9; EE: 31±6; EE+I: 52±7) in EE-induced cholestatic animals. Plasma ALP increased with EE but was not significantly reduced by inulin. While EE tended to decrease BSEP (%) (Control: 94±7, EE:67±8, EE+I: 78±11, I: 88±14) and increase MRP3 (%) (Control: 105±4, EE:157±39, EE+I: 158±63, I: 74±17) there were no significant changes in the EE+I group. Finally, after EE treatment, total liver FXR decreased (%) (Control: 97±2, EE:41±9*, EE+I: 52±10*, I: 82±18), and Inulin treatment did not alter this pattern. **Conclusion.** Inulin supplementation showed a trend toward improved bile flow and bile salt excretion rate in estrogen-induced cholestasis without causing a significant change in transporter expression. The lack of statistical significance in this preliminary study suggests a larger sample size is needed to confirm these findings.

Gast 05

PROTECTIVE EFFECT OF QUERCETIN ON BSEP EXPRESSION AND OXIDATIVE STRESS IN A FRUCTOSE-INDUCED RAT MODEL OF METABOLIC SYNDROME

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Introduction: Metabolic syndrome (MS) is a disorder characterized by obesity, hypertension, dyslipidemia, and insulin resistance, leading to elevated oxidative stress (OS) and inflammation. Bile secretion, a highly regulated process, is affected by inflammatory cytokines and reactive oxygen species (ROS). Previous studies in a rat model of MS demonstrated decreased bile flow and impaired expression and activity of the Mrp2 transporter.

Aims: To assess the protective effect of quercetin (Q), a flavonoid with known antioxidant and anti-inflammatory properties, on the canalicular bile salt transporter Bsep expression in a fructose (F) induced rat model of MS.

Methods: Adult male Wistar rats (220-250 g) were randomly divided into four groups: control (C; n=4), F (n=5, 10% w/v F in drinking water for 8 weeks), F+Q (n=4, Q administered at 15 mg/kg i.p. three times per week for the last 15 days of F treatment), and Q (n=3). Oxidative stress was measured in liver homogenates assessing catalase and superoxide dismutase (SOD) activity, and lipid peroxidation (TBARS). Real-time PCR was performed to evaluate the expression of IL-1β, normalizing the results to the 18S rRNA housekeeping gene using the 2^{-ΔΔCt} method. Bsep expression was analyzed by Western blot from liver homogenates.

Results. (Mean±SEM. **p*<0.05 vs. C; ^x *p*<0.05 vs. F). In the F+Q group, Q reversed the effects of F on oxidative stress. Catalase activity (C): C (100±15), Q (89±20)^x, F (58±13)*, F+Q (91±13)^x. Lipid peroxidation (TBARS): C (100±13), Q (130±15), F (135±20), F+Q (89±25)^x. SOD activity: C (100±13), Q (93±15)^x, F (57±20)*, F+Q (91±25)^x. Quercetin administration also restored Bsep expression to near-control levels, which had been reduced by fructose. Bsep expression: C

(100±25), Q (70±2), F (41±19)*, F+Q (90±30)^x. Finally, Q had no effect on the increase in IL-1 β expression produced by F. IL-1 β expression: C (100±14), Q (173±13), F (256±18)*, F+Q (351±60)*

Conclusion: Quercetin effectively restored antioxidant defenses and Bsep expression, counteracting the oxidative alterations induced by a fructose-rich diet.

Gast 06

MODULATION OF INTESTINAL ABC EFFLUX TRANSPORTERS BY GLIADIN-DERIVED PEPTIDES IN CACO-2 CELLS

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Introduction: ABCC2 and ABCB1 are major intestinal efflux transporters that limit oral drugs bioavailability and food additives toxicity. Gluten-derived gliadins, resistant to complete enzymatic digestion, generate peptides that impair barrier function and activate immune responses. However, data on protein expression and function of ABC efflux transporters in this context are still lacking.

Objectives: To evaluate the effect of gliadin-derived peptides on ABCC2 and ABCB1 expression and function, in Caco-2 cells.

Methods: Caco-2 cells treated with pepsin and trypsin digested gliadin (1 and 5 mg/ml) for 24h. ABCC2 and ABCB1 expression were evaluated by western blot in total cell membranes. The transport activity was determined by quantifying the efflux of dinitrophenyl-S-glutathione (DNP-SG) into the incubation medium (for ABCC2) and the intracellular accumulation of Rhodamine 123 (ABCB1). Proteolysis control includes pepsin and trypsin digested casein.

Results: ABCC2 transport of DNP-SG decreased significantly in both digested gliadin treated groups respect to digested casein and control cells. ABCB1 activity decreased significantly for cells treated with 5 mg/ml of digested gliadin respect to control cells. No significant difference was seen for cells treated with 1 mg/ml of digested gliadin. Protein expression levels of ABCC2 and ABCB1 did not differ significantly between groups.

Conclusion: Exposure to digested gliadin reduces ABCC2 and ABCB1 activity in Caco-2 cells, suggesting that gluten-related disorders may be associated with impaired transporter function, potentially altering the bioavailability and toxicity profile of their substrates.

Gast 07

KIR6.2 KNOCKOUT MICE EXHIBIT REDUCED RECOVERY OF LIVER MASS AND ALTERED BLOOD GLUCOSE LEVELS IN THE PRIMING STAGE OF REGENERATION AFTER PARTIAL HEPATECTOMY

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Introduction: The liver has a unique regenerative capacity to restore its original mass, allowing for safe and easy partial hepatectomy (PH) and partial liver transplantation, which has important implications for the treatment of liver disease. Liver regeneration (LR) is a very complex process that involves cytokines, growth and transcription factors and intracellular signaling events. The metabolic response after PH is an important source of pro-regenerative signals. However, the specific molecular mechanisms by which metabolism controls LR remain poorly understood. Liver expressed-Kir6.2/K-ATP channels are essential in metabolic cellular responses to protect tissue under stress and injury.

Objective: to study the role of Kir6.2 in LR, focusing on the initial metabolic changes after PH.

Methods: Male C57/B6 wild-type (WT) and Kir6.2 knockout (Kir^{-/-}) mice were subjected to glucose tolerance test (GTT). Also, WT and Kir^{-/-} were subjected to two-thirds PH. Blood glucose levels were measured at 0, 1, 2 and 3 h after PH and liver/body weight ratios (LW/BW) were calculated.

Results: GTT showed a peak in blood glucose at 20 min after glucose administration in both groups, with higher levels in Kir^{-/-} (443±43mg/dL) compared with WT (366±13mg/dL). The higher glucose levels in Kir^{-/-} group were maintained thereafter. LW/BW was reduced in Kir^{-/-} after PH (0h WT: 3,63±0,12%; Kir^{-/-}: 3,90±0,08%; 1h WT: 2,04±0,15%; Kir^{-/-}: 1,61±0,10%*; 2h WT:2,16±0,15%; Kir^{-/-}: 1,70±0,07%*; 3h WT: 2,11±0,15%; Kir^{-/-}: 1,58±0,10%*; *p<0.05). Kir^{-/-} mice showed an increase of 65% in blood glucose levels compared with WT at 3 h after PH.

Conclusion: Kir^{-/-} mice exhibit decreased liver proliferation and altered blood glucose levels. Hypoglycemia is described as a metabolic response to PH that promotes liver regeneration. Therefore, the altered glucose homeostasis in Kir^{-/-} mice may explain, at least in part, the reduced recovery of liver mass at the onset of LR.

PREECLAMPSIA, OBESITY, AND FETAL SEX: THE BOUNDARY OF LOW-DOSE ASPIRIN EFFECTIVENESS

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Introduction. Low-dose aspirin (LDA) is administered to pregnant women at risk of developing preeclampsia (PE) to prevent or delay its onset. However, its effect on gestational age (GA) and neonatal outcomes remains unclear. We hypothesize that LDA effectiveness is influenced by maternal obesity and fetal sex.

Objective. To investigate how maternal obesity and fetal sex modulate the response to LDA treatment on neonatal weight in preeclamptic women.

Methods. A retrospective study was conducted at the "Prof. Dr. A. Posadas" National Hospital, including 282 pregnant women, with ethics approval. Participants were classified into eight clinical profiles defined by maternal obesity (BMI<30 kg/m², n=191) or (BMI≥30 kg/m², n=91), LDA administration (150 mg/day from 12 to 36 weeks of gestation), and pregnancy outcome [normotensive (n=237) or preeclampsia (n=45)]. Differences in neonatal weight, analyzed separately for female and male newborns, were assessed using ANOVA followed by pairwise post hoc comparisons with Benjamini–Hochberg correction. To unravel the mechanisms underlying these differences, a moderated mediation analysis was performed to decompose the total treatment effect on neonatal weight, distinguishing whether it was mediated by GA or by fetal growth.

Results In PE obese women, after LDA-treatment, we observed a marked reduction in neonatal weight of female newborns (2320.3 g vs. 3323.3 g; p<0.001 and a reduced GA (–2.6 weeks; p=0.052). Our model indicated that 506.4 g of the reduction in neonatal weight (p=0.049) was attributable to the shorter GA, while an additional 496.5 g decrease was independent of GA (p=0.004), resulting in a total reduction of 1002.9 g (p<0.001). In contrast, male newborns showed no such effect, with both GA and neonatal weight comparable to those observed in normotensive pregnancies.

Conclusion. These findings reveal a sex-specific response to LDA-treatment in PE obese women, with female fetuses particularly susceptible to growth restriction, whereas male fetuses remain unaffected. This highlights the importance of considering both maternal obesity and fetal sex when developing targeted preventive strategies against PE.

Endo 02

DIVING INTO CONGENITAL HYPOTHYROIDISM EFFECTS ON CARDIOVASCULAR FUNCTION

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Introduction It's been widely proven that hypothyroidism (HT) alters cardiovascular function (CF) yet little is known about how the time of inducement would be involved.

Objective The aim of this study was to investigate whether HT induced by methimazole (MMZ) in different stages of life would affect CF.

Materials and methods Sprague-Dawley rats were divided in three groups according to the time they received MMZ: G (free access to water containing MMZ 0,02% from day 9.5 until parturition), GL (free access to water containing MMZ 0,02% from day 9.5 until 21 days after parturition) and C (free access to tap water without MMZ until parturition). After weaning, all male cubs had free access to a balanced diet (Nutrimentos Purina) and water *ad libitum*. Cubs were divided in group A (cubs from G), group B (cubs from GL) and group C (cubs from C, received MMZ 0,02% in the drinking water). Efficacy of MMZ treatment was determined by evaluation of thyroid state. At 90 days, systolic arterial pressure (PAS, mmHg, tail cuff method) and CF (echocardiography) were evaluated. Echocardiographic parameters measured were: heart rate (bpm), left ventricle internal diameter (LVID), anterior and posterior wall thickness (AWT, PWT) in systole and diastole, ejection and shortening fraction (EF, SF). Values are Mean \pm SEM. One Way ANOVA and Kruskal-Wallis test SPSS statistic version 22.0 $P < 0.05$. * versus group C.

Results T3 and T4 plasma levels were increased in groups A and B compared with C group. TSH measurements decreased in both groups compared to group C. Group A and B had higher PAS and HR values than C group. PAS values were similar in all groups while HR increased in A and

B groups. LVID and AWT determinations in systole and diastole were similar between groups. PWT and SF was increased in A and B groups compared with C group. EF increased in group B. **Conclusions** The time in which alterations in the thyroid axis occur is crucial in causing different alterations in CF in adulthood. During the first stages of development, the effect on TSH levels would become more relevant, given that the mother's hormonal contribution seems to compensate for the alteration induced by MMZ.

Endo 03

INULIN PROTECTS AGAINST OBESITY AND INSULIN RESISTANCE VIA ANTIOXIDANT RESPONSE MODULATION IN HIGH-FAT DIET (HFD)-FED MICE

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Introduction: Obesity and insulin resistance predispose to the development of non-alcoholic fatty liver disease (NAFLD). The liver and intestine maintain a bidirectional anatomical and functional communication through the portal circulation. The cause of NAFLD is multifactorial, with insulin resistance, oxidative stress, and an imbalance in the pattern of pro- and anti-inflammatory cytokines—associated with gut microbiota dysbiosis—playing key roles.

In this regard, the intake of prebiotics such as inulin offers a new strategy to treat obesity and related metabolic disorders but mechanisms supporting these associations remain largely unknown.

Objective: This study aims to assess the potential protective role of the prebiotic inulin in the development of obesity and insulin resistance, and to investigate whether such effects are linked to an improvement in oxidative imbalance.

Methods: Adult C57BL/6 wild-type mice (n=3 per group) were fed either a standard diet (CHOW) or a high-fat diet (HFD, 40%) for 16 weeks. Subsequently, mice from both groups received either vehicle or inulin (9.5 g/kg bw/day), via oral gavage three times per week for 4 weeks (CHOW-VEH, CHOW-IN, HFD-VEH, HFD-IN).

Results: Body weight gain was recorded throughout the treatment, showing a significant reduction in HFD mice treated with inulin (% change over the 16 weeks: CHOW-VEH: 100; CHOW-IN: 103±3; HFD-VEH: 143±1*; HFD-IN: 100±2[#]). Blood glucose levels (mg/dl, 12h fasting) were determined: CHOW-VEH: 78±4; CHOW-IN: 102±3; HFD-VEH: 150±10*; HFD-IN: 133±7[#]. The oral glucose tolerance test revealed a reduction in the area under the curve in HFD-IN compared with HFD-VEH animals (p<0.052). To assess oxidative status we determined

Superoxide dismutase 2 (*Sod2*) and hemoxygenase (*Hmox*) by RT-PCR. mRNA levels (% of CHOW-VEH) were *Sod2*: CHOW-IN:119±15; HFD-VEH: 103±20; HFD-IN: 186±4#, and *Hmox* (CHOW-IN:113±14; HFD-VEH: 84±13; HFD-IN: 159±8#) (*p<0.05 vs CHOW-VEH, #p<0.05 vs HFD-VEH).

Conclusion: Our findings suggest that treatment with the prebiotic inulin prevents the development of obesity and insulin resistance. We propose that the modulation of the antioxidant response is a mechanism for these effects.

Endo 04

METABOLIC AND ENDOCRINE ALTERATIONS ASSOCIATED WITH LIFESTYLE IN UNIVERSITY STUDENTS: PRELIMINARY RESULTS

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Introduction University students often adopt unhealthy habits (poor sleep habits, inadequate diet, low physical activity levels, and long study hours), which can trigger oxidative stress and metabolic imbalance, increasing the risk of chronic diseases such as metabolic syndrome, obesity, diabetes, and cardiovascular disorders.

Objectives Evaluate the impact of the diet on college students in terms of oxidative stress, pro-inflammatory responses, and biochemical and endocrine markers.

Methods A validated nutritional survey, anthropometric data, and a standardized physical activity assessment were used. Blood samples were analyzed for metabolic, endocrine, and inflammatory markers.

Results A total of 31 students participated in the study (77% female), of whom 71% reported following an omnivorous diet. No significant differences in BMI, % body fat, % muscle mass, or visceral fat (p>0.05), though vegetarians showed a trend toward healthier body composition. Physical activity levels were moderate. Biochemical analyses revealed values within the reference range for proteinemia, albuminemia, glycemia (GL), insulinemia, HbA1c, uric acid, and thyroid hormones. There was no anemia or leukocytosis. However, 77.3% had estimated GL (eGL) (108.3±5.3 mg/dL), indicating a tendency towards prediabetes and insulin resistance (IR). (HOMA, QUICKI, and TyG indexes). Additional findings included moderate vitamin B12 (B12)

deficiency (32.2%), hyperhomocysteine [12.9% (>16 $\mu\text{mol/L}$), 16.1% (>13.9 $\mu\text{mol/L}$)], and grade 1 subclinical hypothyroidism (25.8%). High-sensitivity C-reactive protein revealed low-grade inflammation [32.2% (1–3 mg/L), 19.3% (>3 mg/L)]. No significant differences were observed between the different dietary groups. However, a healthier body profile was observed in vegetarians.

Conclusion Preliminary findings suggest that metabolic and endocrine alterations, such as IR, B12 deficiency, subclinical hypothyroidism, and inflammation, support the hypothesis that lifestyle factors significantly impact biochemical-endocrine homeostasis.

Endo 05

GRAPE POMACE EXTRACT, RICH IN BIOACTIVE COMPOUNDS, PROTECTS FROM HIGH-FAT DIET-INDUCED OBESITY AND BROWN ADIPOSE TISSUE WHITENING IN MICE.

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Introduction: Excessive accumulation of white adipose tissue (WAT) increases the risk of developing several metabolic and chronic diseases. Unlike WAT, functional brown adipose tissue (BAT) burns energy instead of storing it, playing a crucial role in energy expenditure and thermogenesis.

Objective: We investigated the capacity of grape pomace extract (GPE), rich in polyphenols, to mitigate obesity, subcutaneous and visceral WAT expansion, and BAT dysfunction, as well as the metabolic alterations induced by a high-fat diet (HFD) in mice.

Materials and Methods: Eight-week-old male C57BL/6 mice were randomly divided (7-8 mice/group) into four experimental groups and fed a 60% HFD or a standard diet for 13 weeks, with or without GPE supplementation (300 mg/kg body weight/day). Body weight, adipose tissue remodeling, glucose tolerance, lipid profile, insulin resistance, and plasma lipopolysaccharides (LPS) were evaluated. Levels of BAT markers were analyzed. Statistical analyses were conducted using one-way ANOVA. A p-value < 0.05 was considered statistically significant.

Results: Mice fed a HFD significantly increased body weight gain, adipose tissue mass, adipocyte hypertrophy, BAT whitening, and metabolic alterations such as insulin resistance and plasma LPS compared to the Ctrl and Ctrl+GPE groups. GPE supplementation to the HFD significantly reduced body weight gain and protected against WAT expansion and epididymal

WAT adipocyte hypertrophy. Moreover, GPE attenuated BAT whitening phenotype, preserving tissue morphology and function, as evidenced by reduced lipid accumulation, increased BAT markers, and by a lower WAT/BAT ratio. GPE also decreased metabolic endotoxemia (LPS) and improved insulin sensitivity.

Conclusion: GPE supplementation mitigates HFD-induced obesity, insulin resistance and increased circulating endotoxins, preserving BAT from whitening and maintaining its thermogenic phenotype. These findings support the use of grape-derived bioactive compounds, to counteract obesity-associated metabolic disorders.

Renal physiology

Ren 01

DETECTION OF EXTRACELLULAR VESICLES IN KIDNEY PRESERVATION SOLUTIONS: TOWARDS BIOMARKERS OF GRAFT QUALITY

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Introduction: Delayed graft function (DGF) remains a frequent complication in kidney transplantation, with multifactorial causes involving donor, recipient, and the transplantation procedure itself. Despite continuous advances in the field, the incidence of DGF remains high in Argentina. In 2023, two hypothermic perfusion machines (HPM) were incorporated into national practice, a technology with the potential to increase the use of marginal kidneys, reduce DGF rates, and ultimately improve graft survival. In this context, extracellular vesicles (EVs) have emerged as promising candidates for the identification of non-invasive biomarkers for assessing graft quality and predicting post-transplant function.

Objective: The aim of this study was to initially identify EVs in static preservation solutions and in perfusates from kidneys subjected to HMP, both before and after perfusion.

Methods: Fractions containing EVs were isolated from preservation solutions by differential ultracentrifugation. The isolated EVs were subsequently characterized using three

complementary approaches: Nanoparticle Tracking Analysis (NTA) was performed to determine particle size distribution and concentration; Transmission Electron Microscopy (TEM) was employed to evaluate vesicle morphology; Western blot analysis was conducted to detect the presence of specific EV markers.

Results: Seven samples of solutions from non-perfused kidneys (static preservation: 23.14 ± 4.67 h), 13 samples of pre-perfusion solutions (7.42 ± 2.15 h of preservation), and 13 post-perfusion samples obtained from the transplant centers (10.42 ± 3.34 h in MPH) were analyzed. NTA analysis revealed the presence of particles in all three groups studied. TEM confirmed their morphology and vesicular structure. Western blot detected the presence of EV marker CD63, as well as the expression of AQP2 and NHE3, indicating their renal origin.

Conclusion: Taken together, this initial characterization demonstrates that kidney preservation solutions for transplantation contain EVs of renal origin, which could be explored as a non-invasive source of biomarkers to assess graft quality.

Ren 02

ACTIVATION OF ANGIOTENSIN II TYPE 2 RECEPTOR (AT2R) PREVENTS ACUTE DAMAGE IN RENAL TUBULAR CELLS BY ACTIVATING AUTOPHAGY. ROLE OF THE PRIMARY CILIA.

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Introduction. AT2R activation preserves primary cilia integrity in renal tubular cells subjected to ischemia/reperfusion (IR), eliciting a nephroprotective effect. Cilia-deficient tubular cells are more susceptible to acute kidney injury, partly due to the suppression of nephroprotective autophagy. We reported that AT2R stimulation promotes autophagy in MDCK cells, increasing LC3-II expression.

Objective. To analyze the role of the pro-autophagy effect of AT2R activation in the prevention of IR damage in tubular cells and to evaluate the putative role of primary cilia in that effect.

Methods. Two clones of MDCK cells overexpressing LC3-RFP (A2 and A6) were generated. LC3-II overexpression and reduced p62 protein levels in A2 (-73% , $p<0.05$) and A6 (-78% , $p<0.05$) were verified by Western blot, confirming autophagy activation. Control (C), A2 and A6 cells were cultured under conditions promoting epithelial polarity. IR damage was induced by 90-min incubation in serum-free/ATP-depletion media, followed by 2 h with full media. 24 h before IR, cells were incubated with AT2R agonist C21 ($1\mu\text{M}$). Cell viability was tested by Trypan Blue exclusion ($n=3$). A2 and A6 cells were stained for acetylated α -tubulin to identify cilia, and LC3-II

RFP+ autophagic vesicle area was measured in confocal images. (n=5). Results were analyzed by ANOVA or t-test, and expressed as mean \pm SE. *p<0.05 vs C; #p<0.05 vs basal (B)

Results. LC3-RFP overexpression partly prevented IR damage (C-B: 97 \pm 1; A2-B: 98 \pm 1; A6-B: 98 \pm 1; C-IR: 72 \pm 3#; A2-IR:77 \pm 1*#; A6-IR:80 \pm 1*#) and this effect was not additive to that of C21 (C21-IR: 80 \pm 3#; C21-A2-IR: 76 \pm 3; C21-A6-IR: 79 \pm 3). C21 increased the area of LC3-RFP vesicles in ciliated (C-A2: 0.47 \pm 0.07; C21-C2: 0.56 \pm 0.08*; C-A6: 0.59 \pm 0.07; C21-A6: 0.75 \pm 0.09*), but not in non-ciliated (C-A2:0.55 \pm 0.05; C21-C2:0.57 \pm 0.05; C-A6: 0.68 \pm 0.08; C21-A6: 0.70 \pm 0.05) cells.

Conclusion. Our results show that AT2R stimulation by C21 induces autophagy exclusively in ciliated MDCK cells, and that its protective effect occurs only when autophagy was not already activated by LC3-II overexpression, supporting the cilia's role in C21-autophagy induction and autophagy induction in C21 protective effect.

Ren 03

DEVELOPMENT OF AN EXPERIMENTAL MODEL OF FIBROSIS INDUCED BY UNILATERAL URETERAL OBSTRUCTION IN MICE

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Introduction: Chronic kidney disease (CKD) is a growing global public health concern, driven by population aging and risk factors such as diabetes, hypertension, and obesity. It affects approximately 8–16% of adults and is characterized by the loss of epithelial cells and extracellular matrix accumulation, with fibrosis as its hallmark. Obstructive nephropathy, often associated with kidney stones, increases the risk of CKD by 68%. Animal models of unilateral ureteral obstruction (UUO) are commonly used to study of its pathogenesis and the mechanisms underlying progressive fibrosis.

Objective: To establish an experimental model of fibrosis induced by UUO in mice.

Methods: Adult female mice of the C57BL/6J line were subjected to a surgical procedure in which the left ureter was doubly ligated with surgical silk and then transected between the two ligatures. After 2 weeks, renal damage was evaluated both macroscopically and histologically by preparing

paraffin-embedded kidney tissue sections, followed by hematoxylin-eosin and Picrosirius Red staining.

Results: All mice exhibited macroscopic alterations, with hydronephrosis being evident. Histological changes were observed in 100% of hydronephrotic kidneys, including pelvic-calyceal dilation, urothelial flattening, tubular dilation, and fibrosis, compared to the contralateral non-obstructed kidneys and those from the sham group.

Conclusion: The UUO-induced fibrosis model developed in this study is feasible, reproducible, and provides a valuable tool for the study of obstructive renal pathologies.

Ren 04

RENAL EFFECTS OF FENOFIBRATE IN AN EXPERIMENTAL MODEL OF ESTROGEN-INDUCED CHOLESTASIS

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Introduction: Estrogens can induce intrahepatic cholestasis in susceptible women during pregnancy or hormone therapy. Induction of cholestasis using estrogen 17 α -ethinylestradiol (EE) is a widely used experimental model. Fenofibrate (FF), a selective PPAR α agonist, is used to treat certain cholestatic liver diseases. Our previous studies demonstrated that FF reduces bile salt (BS) accumulation in EE-induced cholestasis by inhibiting BS synthesis and modulating hepatic transporters, including Mrp3 induction and Ntcp downregulation, delivering BS to kidney for alternative urinary excretion.

Objectives: This study aimed to evaluate whether FF induces renal alterations in EE-induced cholestasis.

Methods: Male Wistar rats were randomly assigned into the following groups: i) Control (C), ii) EE (5 mg/kg/day, i.d., 5 days), iii) FF (200 mg/kg/day, p.o. 7 days), and iv) EE+FF. Levels of plasma urea (Up), plasma creatinine (Cp), and urinary creatinine were determined spectrophotometrically. Urine volume was assessed gravimetrically. Glomerular filtration rate (GFR) was calculated by creatinine clearance. Fractional water excretion (FE_{H₂O}) and excreted load of BS (EL_{BS}) were calculated by standard formulas. Total BS in serum and urine were quantified using the Randox[®] kit. Statistical analysis: ANOVA-Newman Keuls, P<0.05: (a) vs C, (b) vs EE, (c) vs FF, (d) vs EE+FF.

Results: Up, Cp, and GFR remained unchanged in all groups. In EE+FF rats, FF reduced serum BS levels to control values, contrasting with the increase induced by EE (+757%^{a,c}). FF increased FE_{H₂O} (+104%^{a,b,c}) and EL_{BS} (+163%^{a,c}) in EE-induced cholestasis.

Conclusion: Renal function, assessed by Up, Cp, and GFR, was preserved. In the kidney, FF promotes an adaptive response to EE-induced cholestasis by promoting urinary BS elimination, resulting in increased FE_{H_2O} and, consequently, a more diluted urine. These changes may be due to adaptive modulation of renal transporters, with upregulation of those mediating BS secretion and downregulation of those involved in BS reabsorption.

Ren 05

CONTRIBUTIONS TO THE SEX SPECIFIC CHARACTERIZATION OF RENAL HANDLING OF NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN IN RESPONSE TO ISCHEMIC ACUTE KIDNEY INJURY

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Introduction. Neutrophil Gelatinase-Associated Lipocalin (NGAL) has emerged as a promising acute kidney injury (AKI) biomarker, rising in plasma (pNGAL) and urine (uNGAL) after renal ischemia-reperfusion (IR). NGAL is filtered through glomerulus, reabsorbed in proximal tubules and synthesized *de novo* and secreted by distal nephrons; thus, uNGAL results from a combination of these processes. In a unilateral IR-AKI rat model, we previously observed increased protein level of NGAL in the ischemic (IK) and contralateral kidney, pNGAL and uNGAL. Some of these results displayed sexual dimorphism. However, the complex landscape of NGAL renal handling in AKI, and its sex-dimorphism implications, needs further exploration.

Objectives. We aimed to contribute to understanding of NGAL renal handling in AKI with a sex specific perspective, focusing on renal expression, cortical reabsorption and extrarenal sources of NGAL.

Methodology. Male (M) and female (F) Wistar rats (n=6 per group) underwent 40 minutes of unilateral renal ischemia and 1 day of reperfusion. Controls underwent sham operation. Kidneys were processed to perform molecular analysis. Renal IL-6, NGAL, and Megalin (an NGAL endocytic receptor) mRNA expression was evaluated in IK using RTqPCR. Hepatic NGAL protein abundance was measured by Western blot. Statistical method: ANOVA post-hoc Tukey test.

Results. After IR, medullary NGAL mRNA was higher in M than F. Cortical expression does not change. Renal IL-6 was upregulated only in M, reflecting a sex-specific local inflammatory

response and female renoprotection. Despite different injury severity, a similar systemic response for both sexes was found since hepatic NGAL levels increased equally in M and F following IR. This suggests the liver could be a possible source of pNGAL. After IR, cortical Megalin expression decreased equally in M and F, which could impair NGAL reabsorption.

Conclusion. We conclude IR-AKI pathophysiology as well as NGAL renal handling during IR-AKI have sex related differences. Although further studies are needed, this work reveals the need to consider sex as a biological variable to achieve better clinical application of NGAL as a biomarker.

Cell Physiology - Signal Transduction

FC 01

MOLECULAR BASIS OF THE POST-TRANSLATIONAL DOWNREGULATION OF INTESTINAL MRP2 BY OXIDATIVE STRESS

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Introduction: The intestinal epithelium is highly susceptible to oxidative stress (OS), which impairs barrier integrity and promotes gastrointestinal dysfunction. Multidrug resistance-associated protein 2 (MRP2) is a major efflux pump in the intestinal transcellular barrier, limiting dietary toxicants absorption. Previously, we confirmed that tert-butyl hydroperoxide (TBH) induced OS at 24 h in Caco-2 cells, and MRP2 expression and activity was significantly decreased at this condition, without changes in mRNA levels.

Objectives: To evaluate the mechanism underlying intestinal MRP2 regulation in Caco-2 cells exposed to OS induced by TBH.

Methods: MRP2 expression was evaluated by western blot in total cell membranes, plasma membranes (PM) and total lysates. Real time RTq-PCR was performed to evaluate changes in mRNA expression. The MRP2 transport activity was determined by quantifying the efflux of dinitrophenyl-S-glutathione (DNP-SG) into the incubation medium by HPLC. Immunofluorescence confocal microscopy was conducted to study MRP2 localization. Statistical analyses were performed using one-way ANOVA followed by the post hoc Tukey-test and results were expressed as a % of control (C).

Results: We confirmed MRP2 internalization after 30 min of TBH treatment, as evidenced by a significant decrease in PM fractions (-50% vs. C, $p < 0.05$; $N=4$) and by the decreased MRP2 apical signal in localization studies in TBH group (-70% vs. C, $p < 0.05$; $N=6$). Treatments with cPKC inhibitor GÖ6976 restored MRP2 localization and activity impaired by TBH. Clathrin mRNA levels were significantly increased (+170%) in TBH group respect to C ($p < 0,05$; $N=4$), while clathrin inhibitor MDC prevented MRP2 reduction by TBH in PM fraction. Treatment with colchicine, a microtubule disruptor, did not revert MRP2 decrease by TBH. Studies with cicloheximide demonstrated that TBH promoted MPR2 degradation, which was restored by proteasome inhibitor MG-132 at 24 h.

Conclusion: Human intestinal MRP2 is downregulated by short-term OS via cPKC- and clathrin-dependent endocytosis and undergoes proteasomal degradation under prolonged OS exposition.

FC 02

DRUG SYNTHESIS, SELECTION AND REPOSITIONING TO PREVENT SHIGA TOXIN-INDUCED RENAL INJURY IN POSTINFECTIOUS HAEMOLYTIC UREMIC SYNDROME

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Introduction: Shiga toxin 2 (Stx2) is an AB5 holotoxin. The B5 subunit mediates cellular entry, while the A subunit, once internalized, targets the ribosome. There, it exerts N-glycosidase activity that results from ribotoxic stress, and ultimately, enhanced apoptosis. Critical residues for this catalytic deamination include Arg170 and Glu167. Clinically, Stx2 represents the major virulence factor of Shiga toxin-producing *Escherichia coli* (STEC), the causative agent of hemolytic uremic syndrome (HUS), most cases are linked to Stx2-producing strains. Currently, no specific treatment is available.

Objectives: Our goal is to identify drugs that bind to Stx2 and prevent the onset of HUS.

Methods: Vero and HK-2 cells were exposed to Stx2 and/or drugs to assess cell viability using a neutral red assay. Steered molecular dynamics simulations were performed with AMBER to investigate potential binding mechanisms of candidate drugs to Stx2A1. The Stx2A1 subunit was

expressed in *E. coli* C43 from a pET28a plasmid and purified using a nickel-affinity column. Protein folding was evaluated by circular dichroism spectroscopy.

Results: Through a virtual screening of 20,000 molecules, we selected nine candidates for this purpose and tested their potential activity in kidney-derived cell lines. In our model, we confirm that cells exposed to Stx2 and treated with B18(FDA-approved) show significantly higher viability than cells exposed to Stx2 without treatment in two cell lines ($p < 0.0001$, $n = 8$, at 10^{-1} μM). Another, FS1 (in-house), which was synthesized, redesigned, and derivatized to improve solubility, also demonstrated to increase viability in cell dependent assays in both cell lines ($p < 0.005$, $n = 8$ at 1 μM).

Molecular dynamics simulations suggest that B18 binds near the residues Arg170 and Glu167 and interacts with Tyr77 and Tyr114 within the druggable pocket, in the Stx2 active site. To explore the antiStx2-drug binding mechanism, Stx2A1 was expressed using a pET28+ plasmid containing the A subunit of Stx2. The proper folding was demonstrated by circular dichroism. Cell-free systems are currently in progress to evaluate the capability of the compounds to bind exclusively Stx2A1.

FC 03

STABILIZATION OF GOLGI-DERIVED MICROTUBULES BY CLASP1/2 PROTEINS IS NECESSARY FOR NK CYTOTOXICITY

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Introduction: Natural killer (NK) cells are cytotoxic lymphocytes that eliminate infected or transformed cells through the formation of an immune synapse (IS). In previous results, we characterized the Golgi apparatus (GA) as a novel microtubule (Mt) organizing center in NK cells involved in LFA-1 accumulation at the IS, which is an essential event in NK cell activation. CLASP1/2 are Mt-associated proteins which stabilize GA-Mt, whose role in NK cytotoxic function remains unclear.

Objectives: To evaluate CLASP1/2 participation in the NK cytotoxic response.

Methods: NK-YTS cells and human NK cells (eNK) isolated from peripheral blood were transduced with lentiviral particles encoding shRNAs targeting CLASP1/2. NK cells were incubated with KT86 target cells. NK-YTS cytotoxic activity was evaluated by determining the % of dead KT86 cells by flow cytometry. LFA-1, lytic granules (LG) and centrosome relocation to the

IS were analyzed by confocal microscopy. LFA-1 accumulation at the IS was calculated as the percentage of total LFA-1-cell fluorescence that was present at the IS. Centrosome polarization was quantified as the distance from the Ct to the IS, minus the distance from the cell centroid to the IS, normalized to the latter. The Area Weighted Distance (AWD) was calculated as the average distance of LG to the IS, weighted by particle area. At least 10 IS were analyzed per experiment. Data are shown as mean \pm SEM from three independent experiments. At least, 10 IS were analyzed per experiment. Results are expressed as media \pm SEM from three different experiments.

Results: Cytotoxic activity was reduced in CLASP knockdown (CLASPKD) YTS-NK cells (Control: 19.75 ± 0.66 ; CLASPKD: 7.21 ; $p<0.05$). LFA-1 accumulation at the IS (Control: $62.73\pm 3.16\%$; CLASPKD: $29.53\pm 5.34\%$; $p<0.05$) and Ct polarization to the IS (Control: -0.41 ± 0.13 ; CLASPKD: 0.33 ± 0.14 ; $p<0.05$) were compromised, and LG AWD to the IS (Control: 1.97 ± 0.15 ; CLASPKD: 3.60 ± 0.68 ; $p<0.05$) was increased in eNK CLASPKD cells.

Conclusion: Our results demonstrate that CLASP1/2 participate in NK cytotoxic activity and highlight the physiological role of GA-Mt in IS maturation.

FC 04

AGRP NEURONS' INVOLVEMENT IN THE PROLONGED OREXIGENIC EFFECT OF GHRELIN: A NEUROANATOMICAL AND FUNCTIONAL ANALYSIS

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Introduction: Agouti-related peptide (AgRP)-expressing neurons of the hypothalamic arcuate nucleus (ARH) are the main population mediating the effects of ghrelin, an orexigenic stomach-derived hormone. Regarded as the core ARH population expressing ghrelin's receptor, GHSR, ablation of AgRP neurons blocks ghrelin's orexigenic effects. Also, ghrelin administration triggers a delayed orexigenic effect concomitant with AgRP neuronal activation. However, a thorough analysis of hypothalamic ghrelin sensing neuronal subpopulations and their involvement in its delayed effects is still missing.

Objectives: To study the distribution of ghrelin-sensing ARH AgRP neurons and their involvement in the long-lasting orexigenic effects of ghrelin.

Methods: We used mice expressing Td-Tomato fluorescent protein in AgRP neurons (AgRP-Tom) centrally or peripherally administered with a fluorescent ghrelin analog to analyze the distribution of AgRP neurons that bind ghrelin in the ARH. We peripherally injected ghrelin in AgRP-Tom mice to assess the distribution of AgRP neurons activated by ghrelin using c-Fos expression. Finally, we peripherally injected ghrelin to mice expressing an inhibitory DREADD in AgRP neurons to analyze their involvement in the prolonged orexigenic effects of ghrelin. We analyzed food intake and c-Fos expression after ghrelin and CNO administration. Comparisons were performed using Student's t-test.

Results: The density of AgRP ghrelin-binding neurons with peripheral or ICV injection was similar in anterior and medial parts of the ARH, but lower in the posterior part for the peripheral route ($p=0.008$). Yet, the distribution of AgRP neurons positive for c-Fos after peripheral ghrelin injection closely matched that of ghrelin binding. Pharmacological inhibition of AgRP neurons 60 min after peripheral ghrelin injection produced a reduced late cumulative food intake (60–180 min, $p=0.016$), with conserved c-Fos activation in the ARH.

Conclusion: AgRP neurons either sensitive to ghrelin or responsive to peripheral ghrelin display a similar distribution in the mouse brain. The blockade of AgRP activity abolishes the prolonged orexigenic effect of ghrelin.

FC 05

ANGIOTENSIN II MODULATION OF NATURAL KILLER EFFECTOR FUNCTIONS. DIFFERENTIAL ROLES OF AT1R AND AT2R

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Introduction. Natural killer (NK) cells are innate lymphocytes that provide early defense against virus-infected or transformed cells. Their effector functions include recognition and elimination of “unhealthy” targets through secretion of cytotoxic molecules and production of cytokines such as IFN- γ . A cell is deemed “unhealthy” when activating signals from damaged-cell antigens prevail over inhibitory signals linked to immune tolerance. Under these conditions, lytic granules (LGs) translocate to the immune synapse (IS) and are released. NK cells express angiotensin II (AngII) receptors AT1R and AT2R. Although AngII has recognized immunomodulatory roles and AT1R antagonism by losartan (Los) shows potential antitumor effects, its impact on NK effector functions remains unclear. We aimed to evaluate the effect of AngII on cytotoxicity and IFN- γ production, and AT1R and AT2R contribution to these effects.

Methods. YTS NK cells were pre-incubated for 24 h with AngII (0.5 μ M), AngII+Los (0.5 μ M, 5 μ M, respectively), or AT2R agonist C21 (1 μ M). IFN- γ production was analyzed by Western blot. Relative density (RD) was calculated as IFN- γ /loading control density. Activation of the cytotoxic response was assessed by co-incubation with erythroleukemia cells for 30 min, and analysis of LG translocation toward the IS by confocal immunofluorescence. Results of three independent experiments were analyzed by ANOVA/Tukey's test and expressed as mean \pm SE, * p < 0.05.

Results AngII showed a non-significant trend toward inhibiting LG translocation, which became significant in AngII+Los and C21 groups (LG–IS distance [μ m]: control: 4.3 ± 0.3 ; AngII: 6.0 ± 0.6 ; AngII+Los: $8.3 \pm 1.1^*$; C21: $8.0 \pm 0.8^*$). IFN- γ levels increased with AngII and C21 (RD / RD control: AngII: $1.7 \pm 0.2^*$; C21: $1.8 \pm 0.3^*$). AngII+Los did not differ significantly from control, AngII, or C21 (1.8 ± 0.8).

Conclusions. AngII via AT2R, prevents activation of the cytotoxic response while promoting an immunomodulatory response mediated by IFN- γ . In contrast, AT1R partially counteracts the AT2R-dependent inhibition of cytolytic activity. The effect of Los on IFN- γ production requires further analysis.

FC 06

ROLE OF CONSTITUTIVE COX-2 EXPRESSION IN THE MODULATION OF P38 MAPK ACTIVATION BY TAUROLITHOCHOLATE (TLC) IN HEPATIC CELLS

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INTRODUCTION: Cholestasis is characterized by intrahepatic bile salt accumulation due to impaired bile flow, leading to cellular damage, inflammation, and apoptosis. Tauroolithocholate (TLC) is a potent cholestatic agent and signalling modulator that activates stress kinases such as p38 MAPK, promoting inflammation and cell death. Cyclooxygenase-2 (COX-2) has been implicated in liver diseases; our group previously showed that its constitutive expression in hepatocytes attenuates cholestatic injury by reducing inflammation and modulating bile salt metabolism. Assessing the effect of COX-2 on the p38 MAPK pathway over time is relevant to understand its role in stress signalling.

OBJECTIVE: To evaluate the role of constitutive COX-2 in regulating p38 MAPK activation by TLC in hepatic cells at different time points.

METHODS: Immortalized murine hepatocytes derived from Wild-Type mice (NCL-V) and from mice with constitutive hepatic COX-2 expression (NCL-C) were used. Cell viability in response to TLC (10, 50 and 100 μ M, 24hs) was analyzed by MTT assay. NCL-V and NCL-C cells were

treated with TLC (5 and 10 μM) for 20 minutes and 3 hours. P38 pathway activation was assessed by Western blot.

RESULTS: In NCL-V cells, TLC induced a dose-dependent reduction of viability: ~21% at 10 μM , ~59% at 50 μM , and ~68% at 100 μM ($p < 0.05$). In NCL-C cells this cytotoxic effect was significantly lower at 10 and 50 μM ($p < 0.05$). Temporal analysis of the p38 MAPK pathway revealed time-dependent differences. At 3 hours, phosphorylation increased significantly at 5 μM in both lines ($p < 0.05$ vs. vehicle) without major differences between them. At 20 minutes, NCL-V cells exhibited a dose-dependent increase, reaching significance at 10 μM ($p < 0.05$ vs. vehicle), whereas NCL-C showed no activation, suggesting attenuation of early p38 MAPK activation by COX-2.

CONCLUSION: Constitutive COX-2 expression reduces the early activation of the p38 MAPK pathway by TLC in hepatocytes, supporting protective role against TLC-induced hepatocellular injury.

FC 07

AQUAPORIN-4 PARTICIPATES IN THE MIGRATION OF RETINAL MÜLLER CELLS BY FACILITATING WATER TRANSPORT AND CYTOSKELETON REORGANIZATION: ROLE OF THE PI3K SIGNALING PATHWAY

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Introduction: In response to retinal injury, the activation of Müller cells injury leads to proliferation, migration and dedifferentiation. We reported that AQP4, the main retinal water channel, contributes to Müller cell proliferation. Although PI3K regulates the cytoskeleton and cell volume, its role in Müller cell migration is still unclear.

Objectives: To evaluate the role of AQP4 in migration of Müller cells and its modulation by the PI3K signaling pathway.

Methods: MIO-M1 Müller cells were exposed to TNG-020 (TGN, AQP4 inhibitor) or 100 nM of Wortmannin (Wort, PI3K inhibitor). We evaluated cell migration (wound healing), AQP4 expression and the cytoskeleton proteins Actin and Vimentin (immunocytochemistry) and the kinetics of cell volume (videomicroscopy) in migrating cells. Statistical analysis: Student's t test.

Results: AQP4 inhibition decreased cell migration by 30% (32 ± 2 vs 23 ± 2 N=4 $p < 0.01$) and reduced water transport in peripheral regions of Müller cells exposed to hypertonic media (15 ± 1 vs 12 ± 1 N=6 $p < 0.01$), without affecting cell volume regulation. In migrating cells, TGN also reduced of the anisotropy (degree of organization) of F-actin fibers (0.35 ± 0.02 vs 0.27 ± 0.03 N=5

$p < 0.01$) and changed Vimentin localization closer to the plasma membrane (0.5 ± 0.1 vs 0.63 ± 0.05 $N=3$ $p < 0.05$). The inhibition of the PI3K pathway reduced the percentage of wound closure and AQP4 expression, without changes in its localization (32 ± 2 vs 26 ± 3 $N=4$ $p < 0.001$). Wort treatment also reduced the anisotropy of actin fibers (0.35 ± 0.02 vs 0.2 ± 0.03 $N=3$ $p < 0.001$) and Vimentin increased its expression and localization at the plasma membrane in comparison to control migrating cells (236 ± 43 vs 349 ± 32 $N=3$ $p < 0.05$ and 0.66 ± 0.03 vs 0.78 ± 0.02 $p < 0.0001$).

Conclusion: We propose that AQP4 participates in Müller cell migration by regulating water flux and cytoskeletal reorganization, being PI3K a modulator of AQP4 the cytoskeleton. Targeting AQP4 may offer a novel strategy to prevent glial scar formation.

FC 08

HUMAN INTESTINAL SPHERIoids: A 3D MODEL FOR STUDYING DIFFERENTIAL ABC TRANSPORTER EXPRESSION AND ACTIVITY DURING THE PROLIFERATION-DIFFERENTIATION TRANSITION

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Introduction: Spheroid culture models (3D) have emerged as advanced systems that more accurately replicate the structural and functional complexity of the intestinal microenvironment. We previously generated a reproducible Caco-2 spheroid model using the Liquid Overlay technique, providing a valuable system for studying the regulation of intestinal ABC transporters, particularly ABCB1.

Objective: To evaluate the temporal expression and activity of ABCB1, as well as its influence on cell viability, in the 3D model vs. the 2D Caco-2 monolayer culture, at 4 and 7 days.

Methods: Caco-2 spheroids were formed by seeding 10,000 cells/well onto 96-well plates coated with 1.5% agarose. After 4 and 7 days of culture, ABCB1 protein expression was analyzed using Western blotting. ABCB1 transport activity was determined by detecting the efflux of doxorubicin (DOX) into the incubation medium. Cell viability (MTT) assays were performed following exposure to DOX at different concentrations and time points. Data were analyzed using one-way ANOVA followed by the post hoc Tukey test, and results were expressed as percentages ($N=4-6$)

Results: ABCB1 protein expression was higher in 3D than in 2D and increased slightly over the culture period. ABCB1 activity was significantly higher in 3D vs. 2D cultures. At 10 μ M DOX, efflux

increased by 41.8% in 3D vs. 2D ($p < 0.001$), with dose-dependent effects (+47.6% at 10 μM vs. 1 μM , $p < 0.001$). The maximum difference was observed between 3D at 10 μM and 2D at 1 μM (+58.5%, $p < 0.001$). At day 7, 3D spheroids maintained significantly higher efflux (+36.7% vs. 2D at 10 μM , +41.7% vs. 2D at 1 μM , $p < 0.001$). MTT assays showed higher viability in 3D vs. 2D after 6 h and 24 h exposure to DOX ($p < 0.001$). At day 7, 3D spheroids preserved viability across 1–50 μM , while 2D cultures exhibited dose-dependent cytotoxicity ($p < 0.01$).

Conclusion: These results validate Caco-2-derived spheroids as a physiologically relevant 3D model for studying ABC transporters. The increased ABCB1 activity and associated resistance to DOX highlight the model's superiority over 2D cultures for investigating drug transport, bioavailability, and resistance.

Oncology – Inflammation

Onco 01

ANALYSIS OF PROTEIN KINASE C ALPHA AND BRAFV600E EXPRESSION AS POSSIBLE PREDICTIVE MARKERS IN THYROID CANCER

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Introduction: Protein kinase C (PKC) plays multiple roles in cancer, and its interaction with BRAF has been described in several tumors. While BRAF inhibition in thyroid cancer (TC) has been studied, the role of PKC α and its combined targeting with BRAF remains unexplored. We previously showed that PKC α is overexpressed in TC cells and biopsies, correlating with reduced survival, and may mediate thyroid hormone-induced proliferation via integrin $\alpha\beta_3$, activating MAPK and PI3K-Akt pathways.

Methods: WRO follicular TC cell viability was evaluated by MTS assay. Protein modulation was measured by Western blot. RNAi transfection was used to knock down BRAF expression. Vemurafenib (PLX) and GF109203X (GF) were used to inhibit BRAF^{V600E} and PKC α , respectively. Anchorage-dependent growth was assessed by colony formation after 14 days. *In silico* analysis of Papillary Thyroid Carcinoma (TCGA, Cell 2014) was performed using CBioPortal. Data were

analyzed by one-way ANOVA from three independent experiments, reporting significant differences when indicated.

Results: TCGA datasets revealed that TC presents the highest frequency of BRAF alterations, mainly the V600E mutation. In BRAF-mutant cells, BRAF knockdown reduced PCNA expression, indicating impaired proliferation. Cell viability assays showed a dose-dependent reduction with PLX, further enhanced by PKC α inhibition ($p < 0.0001$). Dual inhibition also strongly impaired colony formation compared to either treatment alone ($p < 0.001$). Moreover, patient samples harboring BRAF^{V600E} with high PKC α expression displayed increased proliferation mediators and BRAF pathway effectors, reinforcing the therapeutic relevance of combined targeting.

Conclusion: Our results indicate that dual PKC α and BRAF inhibition effectively suppresses tumor proliferation and may serve as both a diagnostic marker and a potential therapeutic strategy, providing alternatives to current treatments.

Onco 02

MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN (MTP) INHIBITION BY THE LIPID-LOWERING AGENT LOMITAPIDE DOES NOT AFFECT LIVER TUMOR DEVELOPMENT IN MICE ON A HIGH-FAT DIET

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Introduction: Lomitapide is a lipid-lowering agent approved for the treatment of homozygous familial hypercholesterolemia (HoFH) through its specific inhibition of microsomal triglyceride transfer protein (MTP). Inhibition of MTP in the hepatocytes by lomitapide disrupts the formation and release of very low-density lipoproteins (VLDL), leading to a subsequent reduction in circulating levels of low-density lipoproteins (LDL). Notably, studies in a chemical hepatocarcinogenesis (CH) mouse model demonstrated that lomitapide administration increased the number of liver tumors and reduced the proportion of normal hepatic parenchyma in treated animals.

Objective: To evaluate the effect of MTP inhibition by lomitapide on HCC development in a dyslipidemia model induced by a high-fat diet (HFD).

Methods: Adult male C57BL/6 mice were subjected to an HQ model and then HFD to induce dyslipidemia. Animals were randomly divided into two groups: control group (vehicle

methylcellulose, oral gavage); lomitapide group (5 mg/kg BW/day, 3 weeks, oral gavage). At the end of the treatment, animals were euthanized, livers were removed and weighed, and visible surface tumors were counted.

Results: No significant differences in liver tumors number were observed between lomitapide-treated ($39,80 \pm 4,188$ N=5) and control ($35,00 \pm 3,421$ N=5) groups ($p=0,401$, Student t-test, significantly different $p<0,05$). Histological analysis showed no major differences in histopathological architecture between the groups, except for a marked increase in hepatic steatosis in the lomitapide group. In an *in vivo* model without metabolic disease, lomitapide reduced VLDL secretion and induces hepatic steatosis accompanied by a marked increase in tumor proliferation. However, in animals with pre-existing metabolic alterations, lomitapide administration improved the evaluated metabolic parameters without exerting notable effects on tumor development.

Conclusion: These results highlight the complexity of drug actions within the same pathology and emphasize the crucial role of the cellular and metabolic environment in determining therapeutic outcomes.

Onco 03

LEUKOTRIENE A4 HYDROLASE: A NOVEL KEY FACTOR HEPATOCELLULAR CARCINOMA PROGRESSION

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Introduction: Leukotriene A4 hydrolase (LTA4H) is the key enzyme in the synthesis of LTB4. Its overexpression was described in various cancers, resulting in LTB4 overproduction and stimulation of cancer cell proliferation. However, its role in liver cancer has not yet been investigated.

Objective: To examine the role of LTA4H in hepatocellular carcinoma (HCC) progression.

Methods: *In silico*: Transcriptomic data from 425 liver samples in TCGA (375 tumors, 50 normal) were normalized to TPM. Expression of LTA4H, LTB4R, and LTB4R2 was compared between normal and HCC tissues and analyzed across stages. Differences were assessed using ANOVA and paired t-tests and results were validated with TIMER 2.0. A Kaplan-Meier survival analysis

was performed between quartiles. *In vivo*: A xenograft model was generated in athymic mice by injecting 5×10^6 Huh7 cells into the flank (n=10). Half received SC-57461A (a selective LTA4H inhibitor, 10 mg/kg) by oral gavage twice weekly for two weeks; the remainder served as controls. Tumor volumes were measured, and PCNA (IHC) and Ki-67 (IF) were evaluated as proliferation markers.

Results: *In silico*: LTA4H, LTB4R, and LTB4R2 were significantly overexpressed in HCC ($p < 2.2 \times 10^{-16}$). This profile persisted through stages I–III, with significant stage-dependent differences ($p < 0.001$), but not in stage IV (LTA4H: $p = 0.072$; LTB4R: $p = 1.000$; LTB4R2: $p = 0.841$). The survival analysis revealed a significant difference between quartiles ($p = 0.002$), and higher LTA4 expression was associated with higher mortality. *In vivo*: SC-57461A treatment reduced tumor size by 68% ($p < 0.01$) relative to controls, with decreased PCNA staining and lower Ki-67 expression.

Conclusion: Our *in silico* findings show that LTA4H and LTB4 receptors are involved in HCC progression during stages I–III, when proliferative activity is predominant. Also, pharmacological inhibition of LTA4H reduces tumor growth by decreasing proliferation of Huh7 cells. This study identifies LTA4H as a key factor in HCC, suggesting its potential as a prognostic biomarker. The findings also highlight the promise of LTA4H inhibitors as a novel approach to develop more effective liver cancer treatments.

Onco 04

MODULATION OF MULTIDRUG RESISTANCE PROTEINS BY THE PHYTOESTROGEN S-EQUOL IN HEPATOCARCINOMA CELLS AND ITS IMPACT ON CHEMOTHERAPEUTIC DRUGS CYTOTOXICITY.

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Introduction: The canalicular transporters P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 (MRP2) are efflux pumps for a wide range of endo- and xenobiotics, including chemotherapeutic drugs. Their expression and activity can be regulated by dietary compounds, hormones, drugs, and other factors.

Objectives: to evaluate the effect of the phytoestrogen S-Equol (SEQ) on the expression and activity of P-gp and MRP2 in the human hepatocarcinoma-derived cell line HuH7 using 2D and 3D models and its impact on drugs cytotoxicity.

Methods: Cells in 2D cultures were incubated with SEQ (1 μ M) or vehicle (DMSO, C, controls) for 48 h. Transporter expression was evaluated at protein and mRNA level through western blot

and qRT-PCR, respectively. Activity was determined measuring Rhodamine123 (Rho123) accumulation and dinitrofenil-glutathione (DNPSG) excretion as P-gp and MRP2 model substrates, respectively. For cytotoxicity assays, cells were incubated with SEQ for 48h, then exposed to Doxorubicin (DOX) (0–500 μ M) for 24h. Cell viability was assessed using the MTT assay in 2D and APH in 3D cultures, and IC₅₀ values were calculated.

Results: A decrease in P-gp (40 \pm 16%) and MRP2 (56 \pm 9%) protein expression was observed compared with controls (100 \pm 3%, n=3, p<0.05), while at mRNA level the reduction was only significant for P-gp (83 \pm 1% vs 100 \pm 3%, n=3, p<0.05). Transport assays showed higher Rho123 accumulation (116 \pm 3% vs 100 \pm 2%, n=3, p<0.05) and reduced DNPSG excretion (91 \pm 2% vs 100 \pm 2%, n=3, p<0.05) in SEQ-treated cells. Cytotoxicity assays revealed decreased IC₅₀ for DOX after SEQ (38 \pm 5 μ M vs 74 \pm 12 μ M, p<0.05). In the 3D model, proliferation rate (72h/48h) decreased significantly (p<0.05) after 500 μ M DOX in SEQ-pretreated cells, but not at concentrations below IC₅₀. APH assays showed lower IC₅₀ in the SEQ group, though not statistically significant (p=0.1).

Conclusion: These findings suggest that SEQ may improve chemotherapeutic response by inhibiting P-gp and MRP2-mediated efflux, thereby increasing intracellular drug accumulation.

Onco 05

MULTICELLULAR TUMOR SPHEROIDS (MCTS): AN *IN VITRO* MODEL FOR STUDYING SORAFENIB RESISTANCE IN HEPATOCELLULAR CARCINOMA (HCC).

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Introduction: Developing more physiologically relevant models is crucial for exploring novel therapeutic strategies against HCC. Multidrug resistance (MDR) markedly limits the efficacy of pharmacological agents such as sorafenib (SFB), a current first-line treatment for HCC. Stromal cells within solid tumors play a key role in both cancer progression and MDR. MCTS, comprising tumor and stromal cells, provide 3D *in vitro* models that better mimic the *in vivo* tumor microenvironment compared to tumor-only spheroids and conventional 2D cultures.

Objectives: To compare viability and migration capacity between MCTS and HCC-only spheroids.

Methods: MCTS consisting of HCC (Huh7), endothelial (EA.hy926), and hepatic stellate (LX-2) cells (1:0.3:0.3 ratio), as well as Huh7-only spheroids, were generated using the liquid overlay

technique. Cultures were either left untreated (DMSO) or treated with SFB (4 or 8 μ M). Viability (APH assay) was assessed on day 4 and after 72 h of treatment. Migration capacity (3D migration assay; area 48h or 72h/area 0h) was evaluated at 48 and 72 h in treated and untreated cultures. HCC-only spheroids were used as the control group.

Results: Compared with HCC-only spheroids, MCTS showed higher viability on day 4 (+33.8%*). After 72 h, no differences were observed between MCTS and spheroids with or without 4 μ M SFB. However, MCTS exhibited greater viability when exposed to 8 μ M SFB (+38.8%*). Untreated MCTS showed higher migration capacity than HCC-only spheroids at both 48 h (+18.1%*) and 72 h (+18.6%*); also 4 μ M SFB-treated MCTS at 48 h (+15.4%*) and 72 h (+15%*). No significant differences in migration were found under 8 μ M SFB. * p <0.05 vs. HCC-only spheroids.

Conclusion: Although further studies are required, our results suggest that MCTS provide a more suitable model for studying HCC progression and therapeutic responses, as they more accurately reproduce tumor–stroma interactions compared to HCC-only spheroids.

Onco 06

DUAL FACES OF HEMEOXYGENASE-1 IN BREAST CANCER: LINKING SUBCELLULAR LOCALIZATION TO TUMOR BEHAVIOR AND DRUG RESPONSE

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Introduction: Breast cancer (BC) is a heterogeneous disease and, specifically, triple-negative breast cancer (TNBC) still lacks targeted treatments. Heme oxygenase-1 (HO-1), a stress-response enzyme, exhibits dual roles: the full-length isoform is enzymatically active and often antitumoral, whereas the truncated nuclear form lacks enzymatic activity and is often pro-tumoral.

Objectives: We aimed to investigate the impact of HO-1 variants: enzymatically active (FL), enzymatically inactive (H25A) and truncated nuclear (T) on BC progression and response to therapy, in hormone-dependent and TNBC cell lines and in TNBC animal model.

Methods: HO-1 variants were stably transfected in T47D, 4T1, and MDA-MB-231 cells. We evaluated proliferation, migration, cytoskeletal organization, and ERK signalling. 4T1 variants were also implanted in BALB/c mice to assess tumor growth and lung metastasis. In addition, pharmacological activation of HO-1 with hemin and its combination with paclitaxel (PTX) or E-64

were analysed. Data were analysed by one- or two-way ANOVA ($p < 0.05$) in triplicate experiments.

Results: FL HO-1 exerted antitumoral effects, reducing proliferation ($21 \pm 2\%$ vs $44 \pm 3\%$ $p < 0.001$), migration ($76 \pm 3\%$ vs $49 \pm 3\%$ $p < 0.001$), and tumor growth (328 ± 201 mm vs 910 ± 544 mm $p < 0.05$). In contrast, the T variant displayed subtype-dependent behaviour: antitumoral in T47D but protumoral in TNBC, enhancing migration ($25 \pm 4\%$ vs $40 \pm 4\%$ $p < 0.01$) and metastatic dissemination ($38 \pm 26\%$ vs $27 \pm 17\%$ $p < 0.01$), despite smaller primary tumors. H25A displayed similarities to T, supporting a role for nuclear HO-1, independent of enzymatic activity. Hemin reduced cell viability ($73 \pm 3\%$ vs $88 \pm 2\%$ $p < 0.001$) and migration ($48 \pm 3\%$ vs $28 \pm 4\%$ $p < 0.001$), and its combination with PTX synergistically enhanced cytotoxicity in 4T1 cells ($65 \pm 2\%$ vs $78 \pm 2\%$ $p < 0.001$). E-64 partially reversed the antitumoral effects of hemin ($82 \pm 4\%$ vs $63 \pm 2\%$ $p < 0.01$), consistent with a requirement of nuclear HO-1 for transcriptional regulation.

Conclusion: HO-1 exerts dual roles in BC depending on subcellular localization and tumor subtype. Pharmacological induction of HO-1, especially in combination with PTX, represents a promising strategy.

Genetics – Gene Therapy

GTG 01

A NOVEL TRUNCATED ISOFORM OF THE HUMAN TGF- β TYPE II RECEPTOR FC-TAG PROTEIN REVERSES LIVER FIBROSIS BY INDUCING HEPATIC METABOLIC REPROGRAMMING IN RATS

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Introduction: Chronic liver diseases are characterized by an excessive wound-healing response that leads to liver fibrosis. TGF- β signaling is enhanced in fibrosis. Thus, it has become a promising therapeutic target to assist in the recovery of liver function. Previously, we showed that the T β RII-SE/Fc fusion protein exerts a robust prophylactic effect on liver fibrogenesis.

Objective: In this work we aimed to evaluate the mechanisms involved in the therapeutic effect of T β RII-SE/Fc in an *in vivo* model of chronic wound healing.

Methods: We evaluated the effect of intrahepatic administration of a lentiviral vector encoding T β RII-SE/Fc in a rat CCl₄-induced chronic liver injury model. Experimental groups were designed as follows: the control group received CCl₄ vehicle; the CCl₄ group received CCl₄ for 10 weeks; the Lv.T β RII-SE/Fc + CCl₄ group received CCl₄ for 10 weeks; and the Lv.T β RII-SE/Fc at week 4 (n=4-5). Statistical differences among groups were performed using two-way ANOVA. For all analyses, a p-value < 0.05 was considered statistically significant.

Results: In rat livers, T β RII-SE/Fc administration reduced CCl₄-induced Col1A1 (p<0.001), TGF- β 1 (p<0.001), TGF- β 2 (p<0.001) and TGF- β 3 (p<0.001) mRNA expressions together with a reduction of α -SMA (p<0.05) at protein and mRNA levels. Moreover, by modulating lipid-related genes, T β RII-SE/Fc enhanced hepatic triglyceride levels (p<0.001) and restored fatty acid oxidation (p<0.01).

Conclusions: In the liver, T β RII-SE/Fc modulates lipid metabolism to restore homeostasis after chronic damage. Additionally, T β RII-SE/Fc induced metabolic reprogramming to enhance liver regeneration. T β RII-SE/Fc fusion protein might represent an antifibrotic treatment approach in chronic liver diseases.

GTG 02

T β RII-SE/Fc, A PAN TGF- β INHIBITOR, REDUCES LIVER STEATOSIS MODULATING LIPID METABOLISM IN A METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE RAT MODEL

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease worldwide. Lipid accumulation led to the activation of TGF- β signaling pathways, promoting metabolic dysregulation. Nowadays, treatments for MASLD patients are limited. Recently, we have described T β RII-SE, a human T β RII splice variant

encoding a soluble isoform. We fused T β RII-SE with the human IgG-Fc domain, making T β RII-SE/Fc. We have demonstrated that lentiviral-mediated liver overexpression of T β RII-SE/Fc dramatically decreased microvesicular steatosis in a rat model of MASLD.

Objectives: Here, we aimed to evaluate how T β RII-SE/Fc delivered by lentiviral vectors (Lv. T β RII-SE/Fc) to the liver attenuates liver steatosis in a western diet (WD)-induced MASLD rat model.

Methods: We compared three groups: control diet, WD, and WD+Lv.T β RII-SE/Fc delivered by intrahepatic injection at week 10. In week 21, animals were sacrificed, and livers were stored at -80°C for molecular analysis by RT-qPCR.

Results: We found that T β RII-SE/Fc upregulated PPAR α ($p < 0.05$) and CPT1a ($p < 0.01$) expression (linked to β -oxidation). On the other hand, we observed downregulation of CD36 mRNA ($p < 0.05$) (involved in free fatty acid uptake) and DGAT-2 mRNA ($p < 0.05$) (the main component in lipid droplet formation). However, VLDL synthesis remained unchanged, with no differences in MTTP gene expression.

Conclusion: In this MASLD model, T β RII-SE/Fc promotes β -oxidation and decreases lipid uptake and triglyceride synthesis in hepatocytes. Together, these effects contribute to reducing liver steatosis and highlight the importance of using T β RII-SE/Fc to reverse MASLD.

Immunology – Neuroimmunoendocrinology

INM 01

IMMUNE DYSREGULATION IN AUTOIMMUNE HEPATITIS: TH17/TREG PROFILE AND IMPACT OF THERAPY

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Introduction: Autoimmune hepatitis (AIH) is an inflammatory liver disease of unknown etiology that, if unrecognized or untreated, may progress to cirrhosis and end-stage liver failure. Its clinical presentation is variable, ranging from acute and even fulminant hepatitis to insidious or asymptomatic forms, making early diagnosis challenging. Although immunosuppressive therapy achieves remission in some patients, the immunological mechanisms that sustain disease activity and contribute to relapse remain incompletely understood. CD4⁺ T lymphocyte subsets, including

Th17, regulatory T cells (Treg), are key regulators of immune balance, yet their role in AIH pathogenesis and treatment response has not been fully elucidated.

Objective: To investigate the distribution of circulating Th17 and Treg cells in patients with autoimmune hepatitis, and to evaluate their association with disease activity and the effect of immunosuppressive therapy.

Methods: Adult AIH patients under follow-up at the Hospital Provincial del Centenario Rosario were enrolled. Two groups were defined: untreated AIH (AIHwT, n=5) and AIH under therapy with prednisone (10 mg/day) plus azathioprine (50–100 mg/day) (AIHT, n=20). An age-matched group of healthy individuals was included as controls (Co, n=27). Peripheral blood was collected; IgG levels, autoantibodies was determined. Th17 and Treg subsets were quantified by flow cytometry.

Results: Compared with controls, AIH patients showed elevated IgG levels and autoantibodies ($p<0.05$), consistent with persistent humoral autoimmunity. Untreated patients displayed a pronounced proinflammatory profile, with increased Th17 frequencies (Co: 10.1 ± 0.6 ; AIHwT: $15.5\pm 2.3^*$, AIHT: 10.6 ± 1) and expanded Treg (Co: 6.4 ± 0.3 ; AIHwT: $9.3\pm 1.3^{*\#}$, AIHT: 5.9 ± 0.6) ($*p<0.05$ vs Co, $\#p<0.05$ vs AIHT), suggesting a compensatory mechanism.

Conclusions: Our findings indicate that AIH involves profound dysregulation of effector and regulatory CD4⁺ T cell compartments, and that persistence of humoral autoimmunity may contribute to chronicity and relapse. These insights reinforce the need for immunomonitoring and support the development of targeted therapies to restore tolerance in AIH.