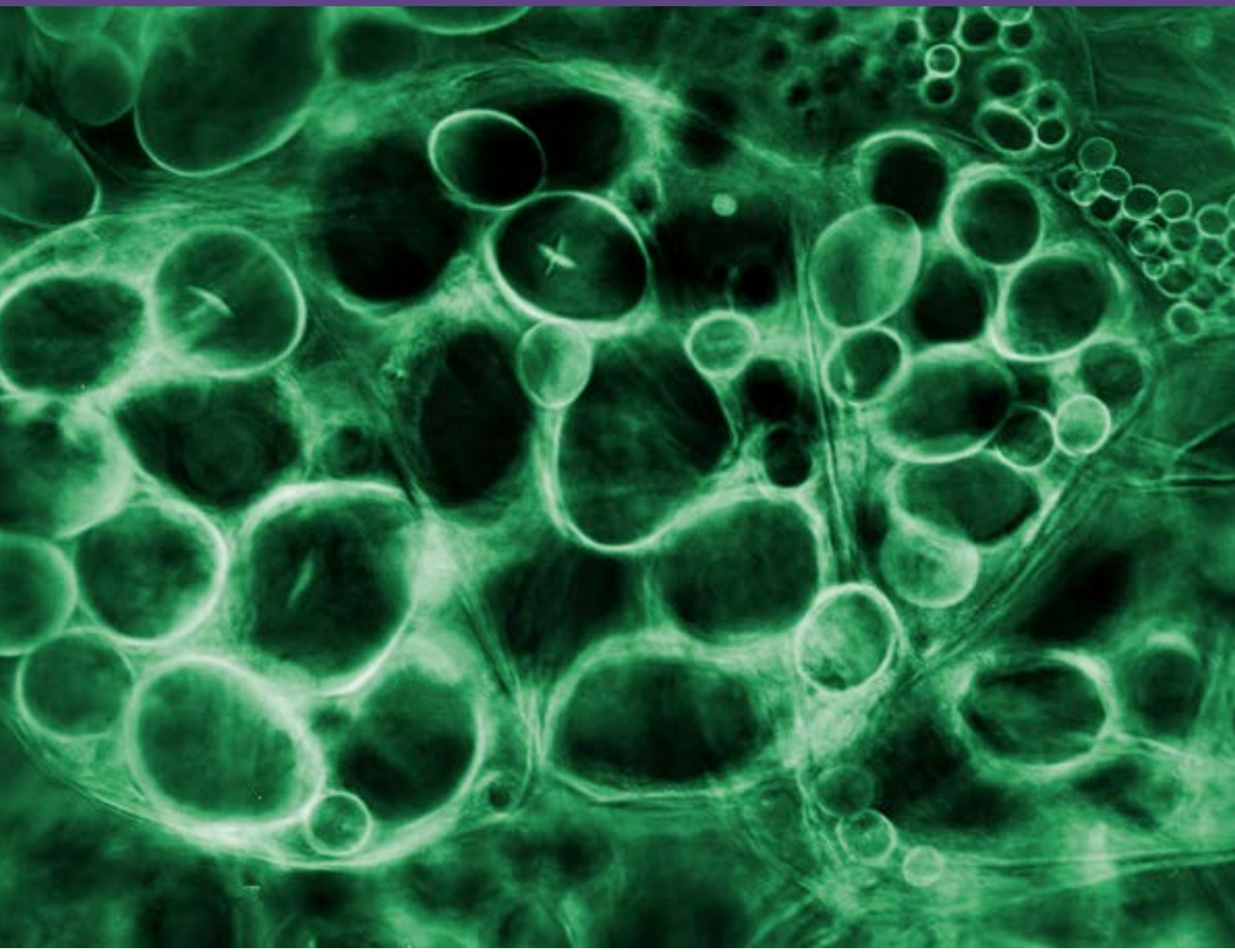
Physiological Mini Reviews

Special Issue
PANAM 2023
PHYSIOLOGICAL SCIENCES
PROGRAM AND ABSTRACT BOOK

16
Volume



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Physiological Mini Reviews

PANAM 2023

Physiological Sciences

Hosted by Chilean Society of Physiological Sciences &
Latin American Association of Physiological Sciences

November 27-30, 2023
Puerto Varas, Chile

Part of the 'Year of Physiology' IUPS

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Program and Abstract Book

PANAM Physiological Sciences 2023

November 27th-30th 2023

Puerto Varas, Chile

PANAM Executive Committee Welcome Letter

."... Bajo los volcanes, junto a los ventisqueros, entre los grandes lagos, el fragante, el silencioso, el enmarañado bosque chileno... Quién no conoce el bosque chileno, no conoce este planeta. De aquellas tierras, de aquel barro, de aquel silencio, he salido yo a andar, a cantar por el mundo."

"…*Under the volcanoes, next to the snow drifts, among great lakes, the fragrant, the silent, the tangled Chilean Forest…Who does not know the Chilean forest, does not know this planet. From those lands, from that mud, from that silence, I have gone out to walk, to sing around the world*."

"El bosque chileno" ("The Chilean Forest"), Pablo Neruda.

Dear colleagues,

We would like to give you a warm welcome to Puerto Varas, Chile. We are sure that you will find excellent science in this Congress, as experienced in the two previous PANAM Meetings; but we are also sure that you will get to discover a beautiful city, surrounded by amazing Patagonia landscape.

You will enjoy an interesting scientific program which will cover the full range of cutting-edge physiology research. We have curated an exciting program consisting of 20 conferences, 32 symposia, 12 workshops, 2 pre-congress courses, 3 activities for and with the community, 2 satellite symposia, and 1 scientific contest. We have convoked referent speakers for all of these activities, involving colleagues from 22 LAC/Eur/Asia countries. You will also have the chance to attend the presentation of original scientific and educational communications, which will be presented both as oral or poster presentations. We hope that these activities can promote fruitful interaction between young and senior investigators.

We are also glad to mention that our Meeting was selected by the International Union of Physiological Sciences (IUPS) as part of the program "Year of Physiology". As members of the American physiological societies, we can take pride in the fact that our region has become the center of attention for physiologists and researchers in related fields from all around the world.

Welcome again to the PANAM Meeting; you are formally invited to combine the passion for unveiling the secrets of physiology with the fun of discovering the lakes, the volcanos and the colonial architecture of Puerto Varas, a South-American pearl that deserves to be enjoyed.

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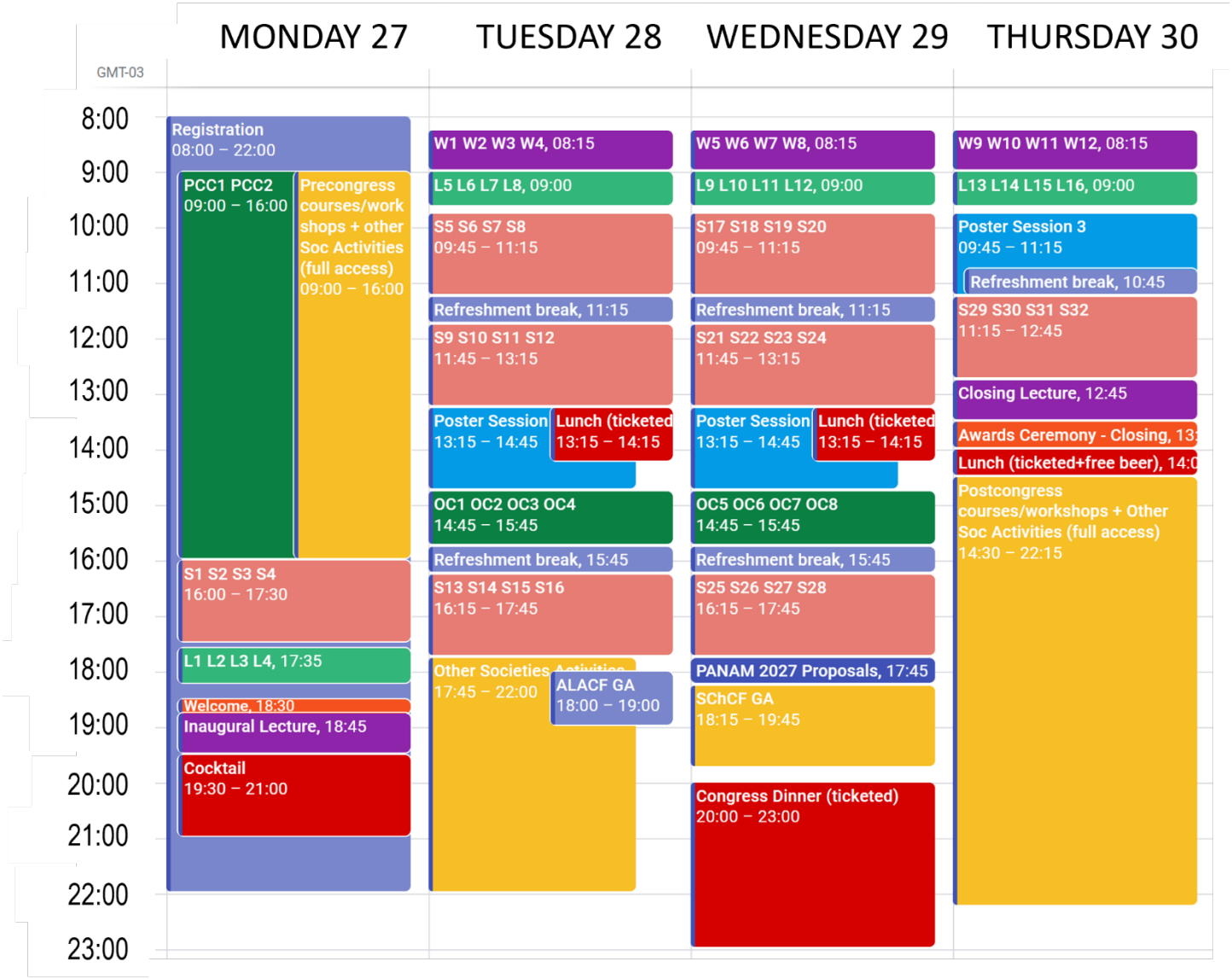
Program at glance



Scientific Program

Friday 24 November 2023 (activities in Santiago de Chile)

11:00	**Satellite symposia (SS) (Santiago de Chile)** ***In memory of Prof Enrique Forero***	
SS1	Role of the International Council of Sciences and Academies of Sciences in academic development. (Sponsored by the National Academy of Sciences of Honduras, Embassy of Honduras to Chile, and International Science Council Regional Focal Point for Latin America and the Caribbean, ISC RFP-LAC)	PUC
15:00	**Satellite symposia (SS) (Santiago de Chile)** ***In memory of Prof Enrique Forero***	
SS2	Dialogue "Science in LAC" with the Chilean Academy of Sciences (AChC). (Sponsored by the AChC, the National Academy of Sciences of Honduras, Embassy of Honduras to Chile, and ISC RFP-LAC)	AChC

Monday 27 November 2023

09:00	**Pre-congress courses (PCC)**	
PCC1	Shaping the future of skeletal muscle: methodologies and emerging findings	Room 1
PCC2	Pre-congress teaching one day workshop. (Sponsored by ADInstruments)	Room 2
16:00	**Symposia (S)**	
S1	Extracellular vesicles for diagnosis and therapy	Room 1
S2	Obesity: challenge of the future	Room 2
S3	Overview of placenta – brain axis in pregnancy complications	Room 3
S4	The intersection of metabolic and inflammatory mechanisms underlying cardiovascular disease; emerging evidence of sex differences	Room 4
17:35	**Lectures (L)**	
L1	Generative artificial intelligence in medicine. (Sponsored by the National Academy of Sciences of Honduras and the Embassy of Honduras to Chile) **Mario Lanza Santamaría** – National Academy of Sciences of Honduras, Honduras	Room 1
L2	Operating principles of the brain oxytocin system **Valery Grinevich** – Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, Heidelberg University, Germany	Room 2
L3	Contextual tissue image cytometry using AI-empowered precision microscopy (Sponsored by URSULAB Chile) **Rupert Ecker** – University of Queensland, Australia	Room 3
L4	Skeletal muscle atrophy and age-related loss of muscle mass and function **Sue C. Bodine** – Aging and Metabolism Research Program, Oklahoma Medical Research Foundation, USA	Room 4
18:30	**Opening Ceremony**	Plenary
18:45	**Inaugural Lecture** The Fluid–Mosaic Membrane Model and membrane lipid replacement **Garth L. Nicolson** – Institute for Molecular Medicine, Huntington Beach, USA	Plenary

19:30	**Welcome cocktail** Coffee sponsored by the Embassy of Honduras to Chile	Terrace
21:30	**End of Day One**	

Tuesday 28 November 2023

08:15	**Workshops (W)**	
W1	Workshop physiology and narratives	Room 1
W2	Tissuegnostics. (Sponsored by URSULAB Chile)	Room 2
W3	Precision in breathing, a workshop on small animal adult and neonate plethysmography	Room 3
W4	Neural orbit (the NEO project): implementing new physiological technologies to motivate new generations of physiologists	Room 4
09:00	**Lectures (L)**	
L5	Titin: big protein with big responsibilities **Henk Granzier** – University of Arizona, USA	Room 1
L6	Neuropeptide neurotransmission in stress physiology in brain and periphery **Lee E. Eiden** – Section on Molecular Neuroscience, National Institute of Mental Health, USA	Room 2
L7	Nitric oxide: a major regulator of the beginning and the end of microvascular permeability in inflammation **Walter N. Durán** – New Jersey Medical School, USA	Room 3
L8	Thermoregulation in extreme environments – lessons learned from studies in sub-Sahara and space **Hanns-Christian Gunga** – Charité-Universitätsmedizin Berlin, Institute of Physiology, Center for Space Medicine and Extreme Environments Berlin, Germany	Room 4
09:45	**Symposia (S)**	
S5	Cross talk between different organs	Room 1
S6	New horizons in cardiorenal ion transport	Room 2
S7	Novel aspects of cell communication in the microcirculation	Room 3
S8	Lipid metabolism and adipose tissue in eating behaviour and metabolic regulation (Sponsored by ELSEVIER)	Room 4
11:15	**Refreshment Break**	Terrace
11:45	**Symposia (S)**	
S9	Hot topics in chronic kidney disease	Room 1
S10	Physiological and molecular regulation of the hypothalamic-neurohypophysial system	Room 2
S11	Muscle-organ crosstalk: focus on diseases	Room 3
S12	Heart failure: more than a cardiac disease	Room 4
13:15	**Poster Session 1 & Lunch (ticketed) with free beer**	Terrace
[14:00]	Technical Workshop 1 (TW1) – *The Journal of Physiology*	Terrace
14:45	**Oral Communications (OC)**	
OC1	Cardiovascular and respiratory	Room 1
OC2	Renal and gastrointestinal	Room 2
OC3	Skeletal muscle and exercise physiology	Room 3
OC4	Lipids and nutrition	Room 4
15:45	**Refreshment Break**	Terrace
16:15	**Symposia (S)**	
S13	New physiological and therapeutic frontiers of the intra-renal renin angiotensin system	Room 1

S14	Mining the waveform, new approaches to delineating respiratory features and analyzing respiratory outcomes	Room 2
S15	Obesity and the risk of cardiometabolic diseases (Sponsored by ELSEVIER)	Room 3
S16	Recent advances in the physiology of the immune system in health and disease	Room 4
18:00	**ALACF General Assembly**	Room 1
19:00	**End of Day Two**	

Wednesday 29 November 2023

08:15	**Workshops (W)**	
W5	Best practices for publishing in the American Journal of Physiology – Renal Physiology	Room 1
W6	Leveraging educational technology in physiology education	Room 2
W7	Generative artificial intelligence for teachers (Sponsored by the National Academy of Sciences of Honduras and the Embassy of Honduras to Chile)	Room 3
W8	Didactics of sciences applied to the teaching of physiological sciences	Room 4
09:00	**Lectures (L)**	
L9	Competency-based education in physiology **Dee Silverthorn** – Dell Medical School, University of Texas at Austin, USA	Room 1
L10	Amyloid-β and tau: the trigger and bullet in Alzheimer's disease pathogenesis **George Bloom** – University of Virginia, USA	Room 2
L11	The central respiratory pattern generator and control of the ventilation: new pathways and players **Jan-Marino Ramírez** – University of Washington School of Medicine, USA	Room 3
L12	Incretin regulation of the hypothalamic-neurohypophysial system **David Murphy** – Bristol Medical School, Translational Health Sciences, University of Bristol, UK	Room 4
09:45	**Symposia (S)**	
S17	Peptide modulation in systems physiology	Room 1
S18	Connecting students with the community to enhance learning	Room 2
S19	Recent advances and future avenues in understanding obesity as a premature aging phenotype. NOTE: *S19 Cancelled by the Chairs (Nov 14, 2023)*	Room 3
S20	New pathological mechanisms of cardiovascular diseases	Room 4
11:15	**Refreshment Break**	Terrace
11:45	**Symposia (S)**	
S21	New insights into the study of adaptive and maladaptive myocardial growth	Room 1
S22	Nociception and pain: from mechanisms to therapeutic approaches	Room 2
S23	Pathophysiology signalling mechanisms in diseases	Room 3
S24	Role of the immune system in hypertension and diabetes	Room 4
13:15	**Poster Session 2 & Lunch (ticketed) with free beer**	Terrace
[14:00]	Technical Workshop 2 (TW2) – *Bentham Science*	Terrace
14:45	**Oral Communications (OC)**	
OC5	Immunology and cancer	Room 1
OC6	Endocrine and metabolism	Room 2
OC7	Education	Room 3
OC8	Neurophysiology	Room 4

15:45	**Refreshment Break**	Terrace
16:15	**Symposia (S)**	
S25	Hormonal signalling in cardiovascular disease	Room 1
S26	Hormones and control of breathing	Room 2
S27	Vascular dysfunction with Covid-19	Room 3
S28	Molecular and physiological mechanisms of the early programming of diseases	Room 4
17:45	**PANAM Physiological Sciences 2027 Proposals Session**	Room 1
18:15	**SCHCF General Assembly**	Room 1
19:00	**End of Day Three**	
20:00	**Conference Dinner**	Bellavista

Thursday 30 November 2023

08:15	**Workshops (W)**	
W9	Exploring gamified pedagogical approaches for neurophysiology in educational spaces	Room 1
W10	Scientific competition session in physiological sciences for undergraduate students PANAM 2023	Room 2
09:00	**Lectures (L)**	
L13	From the laboratory bench to the patient: pre-clinical and clinical development of a drug for obesity and type II diabetes **Carlos Escande** – Institut Pasteur de Montevideo, Uruguay	Room 1
L14	Hypertension, diabetes, and the kidney **Victoria Velarde** – Universidad de Valparaíso, Chile (Sponsored by the Chilean Society of Physiological Sciences)	Room 2
L15	Leptin and control of breathing **Vsevolod Polotsky** – Department of Anaesthesiology and Critical Care Medicine, George Washington University SMHS, USA	Room 3
L16	Nuclear positioning and mechanotransduction in health and disease **Gregg G. Gundersen** – Columbia University, USA	Room 4
09:45	**Poster Session 3**	Terrace
[10:15]	Technical Workshop 3 (TW3) – *ELSEVIER*	Terrace
10:45	**Refreshment Break**	Terrace
11:15	**Symposia (S)**	
S29	Channels and membrane transport in diseases	Room 1
S30	Chemoreceptors in health and disease: exploring new avenues of treatment	Room 2
S31	Myofilament-based mechanisms of muscle disease	Room 3
S32	Tba	Room 4
12:45	**Closing Lecture**	Terrace
	Cardiac CaMKII: Past, Present, and Future - Tracing the journey from our laboratory's experience. (Sponsored by ALACF) **Alicia Mattiazzi –** Cardiovascular Research Center, Faculty of Medicine, Universidad de La Plata, CONICET, Argentina	Plenary

13:30	**Awards Ceremony**	Plenary
14:00	**Lunch (ticketed) with free beer**	Terrace
14:00	**Close of Conference**	

Activities Details

INAUGURAL LECTURE
Garth L. Nicolson (Institute for Molecular Medicine, Huntington Beach, USA)
The Fluid–Mosaic Membrane Model and membrane lipid replacement

ALACF CLOSING LECTURE
Alicia Mattiazzi (Cardiovascular Research Center, Faculty of Medicine, Universidad de La Plata, CONICET, Argentina)
Cardiac CaMKII: Past, Present, and Future - Tracing the journey from our laboratory's experience

LECTURES

L1

Embassy of Honduras in Chile Lecture
Advances in the Application of Artificial Intelligence in Medicine
Mario Lanza Santamaría • *National Academy of Sciences of Honduras.*

L2

Principles of neuropeptide signalling in the brain: oxytocin as an example
Valery Grinevich • *Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, Heidelberg University, Germany.*

L3

URSULAB Chile Lecture
Cell and tissue imaging
Rupert Ecker • *University of Queensland, Australia.*

L4

Skeletal muscle atrophy and age-related loss of muscle mass and function
Sue C. Bodine • *Aging and Metabolism Research Program, Oklahoma Medical Research Foundation, USA.*

L5

Titin: big protein with big responsibilities
Henk Granzier • *University of Arizona, USA*.

L6

Neuropeptide neurotransmission in stress physiology in brain and periphery
Lee E. Eiden • *Section on Molecular Neuroscience, National Institute of Mental Health, USA.*

L7

Nitric oxide: a major regulator of the beginning and the end of microvascular permeability in inflammation
Walter N. Durán • *New Jersey Medical School, USA.*

L8

Thermoregulation in extreme environments – lessons learned from studies in sub-Sahara and space

Hanns-Christian Gunga • *Charité-Universitätsmedizin Berlin, Institute of Physiology, Center for Space Medicine and Extreme Environments Berlin, Germany.*

L9

Education in physiology

Dee Silverthorn • *Dell Medical School, University of Texas at Austin, USA*.

L10

Amyloid-β and tau: the trigger and bullet in Alzheimer's disease pathogenesis

George Bloom • *University of Virginia, USA.*

L11

The central respiratory pattern generator and control of the ventilation: new pathways and players

Jan-Marino Ramírez • *University of Washington School of Medicine, USA.*

L12

Incretin regulation of the hypothalamic-neurohypophysial system

David Murphy • *Bristol Medical School, Translational Health Sciences, University of Bristol, UK.*

L13

From the laboratory bench to the patient: pre-clinical and clinical development of a drug for obesity and type II diabetes

Carlos Escande • *Institut Pasteur de Montevideo, Uruguay*.

L14

Chilean Society of Physiological Sciences Lecture

Hypertension, diabetes, and the kidney

Victoria Velarde • *Faculty of Sciences, Universidad de Valparaíso, Chile.*

L15

Leptin and control of breathing

Vsevolod Polotsky • *Department of Anaesthesiology and Critical Care Medicine, George Washington University SMHS, USA.*

L16

Nuclear positioning and mechano-transduction in health and disease

Gregg G. Gundersen • *Columbia University, USA.*

L17

International Science Council RFP-LAC Lecture

The role of the International Science Council for the progress of science and knowledge in the Americas

Salvatore Aricò • *International Science Council (ISC), France*.

**Santiago de Chile, Nov 24, as part of the satellite symposium 1 (SS1).*

SATELLITE SYMPOSIA (activities in Santiago de Chile)

SS1

Role of the International Science Council and Academies of Sciences in Academic Development

Date: *Friday November 24, 2023*
Place: *Pontificia Universidad Católica de Chile – UC, Sala Colorada, Centro de Extension, Avenida Libertador Bernardo O'Higgins 340,* Santiago *de Chile*
In memory of Prof Enrique Forero 🎗
Sponsored by the International Science Council RFP-LAC (ISC RFP-LAC), the Chilean Academy of Sciences, The Embassy of Honduras in Chile, and the National Academy of Sciences of Honduras

11:00 **Welcome**
Luis Sobrevia • *UC Medicine, ISC RFP-LAC, Latin-America Academy of Sciences (ACAL).*
11:05 **Greetings from Authorities**
Linda Redondo • *Ambassador of Honduras in Chile.*
Pedro Bouchon, Vice Rector of Research, UC • *UC Authorities.*
11:20 **Invited lecture: The role of the International Science Council for the progress of science and knowledge in the Americas**
Salvatore Aricò • *CEO International Science Council (ISC).*
11:50 **Networking**
12:15 **Invited lecture: Generative artificial intelligence applied to education**
Mario Lanza Santamaría • *President of the National Academy of Sciences of Honduras*
12:50 **Q&A**
13:00 **Closing**

SS2

Dialogue Science in Latin America and the Caribbean (LAC) with the Chilean Academy of Sciences

By invitation only
Date: *Friday November 24, 2023*
Place: *Academia Chilena de Ciencias (AChC), Almirante Montt 454 - Santiago - Chile*
In memory of Prof Enrique Forero 🎗
Sponsored by the Chilean Academy of Sciences, International Science Council RFP-LAC (ISC RFP-LAC)

15:00 **Introduced by Cecilia Hidalgo** • *President of the Chilean Academy of Sciences.*
Participants:
Linda Redondo • *Ambassador of Honduras in Chile.*
Salvatore Aricò • *CEO International Science Council (ISC).*
Mario Lanza Santamaría • *President of the National Academy of Sciences of Honduras.*
Luis Sobrevia • *UC Medicine, ISC RFP-LAC, ACAL.*
16:00 **Closing**

SYMPOSIA

S1 - EXTRACELLULAR VESICLES FOR DIAGNOSIS AND THERAPY

Chairs: Patricia Rocco (Federal University of Rio de Janeiro, Brazil), Maroun Khoury (Universidad de los Andes, Chile)

Extracellular vesicles: New challenges to understanding and treating diseases
Ana Claudia Trocoli Torrecilhas • *UNIVESP, Brazil.*

Extracellular vesicles are source of prognostic markers in head and neck cancer
NOTE: *This talk was cancelled by the chair (Nov 23, 2023)*
Adriana Paes Leme • *CNPEM, Brazil.*

Therapy with extracellular vesicles in respiratory diseases
Patricia Rocco • *Federal University of Rio de Janeiro, Brazil.*

S2 - OBESITY: CHALLENGE OF THE FUTURE

Chairs: M Alicia Carrillo-Sepulveda (New York Institute of Technology, USA), Jennifer Thompson (University of Calgary, Canada)

Obesity and its vascular complications
M Alicia Carrillo-Sepulveda • *Department of Biomedical Sciences, New York Institute of Technology, USA.*

Obesity and kidney injury: albuminuria and pathogenesis of diabetic kidney disease
Celso Caruso Neves • *Federal University of Rio de Janeiro, Brazil.*

Skeletal muscle atrophy and mitochondrial dysfunction in a heart failure with preserved ejection fraction-obesity model
Bianca Daniela Nieblas León • *School of Medicine and Health Sciences, Tecnologico de Monterrey, México.*

Maternal obesity is associated with metabolic endotoxemia and changes in the phenotype of hematopoietic progenitor cells and monocytes of their offspring at birth
Macarena Lépez • *Faculty of Medicine, Pontificia Universidad Católica de Chile & Faculty of Medicine, Universidad de Chile.*

S3 - OVERVIEW OF PLACENTA – BRAIN AXIS IN PREGNANCY COMPLICATIONS

Chair: Carlos Escudero (Universidad del Bío-Bío, Chile)

Impaired brain angiogenesis in offspring from preeclampsia. What we have learnt from preclinical models
Carlos Escudero • *Universidad del Bío-Bío, Chile.*

Placenta and maternal mental health during COVID-19 pandemic. Focus in Latin America
Marcelo González Ortiz • *Universidad de Concepción, Chile.*

Potential mediators involved on the cerebrovascular complications of preeclampsia
Pablo Torres-Vergara • *Universidad de Concepción, Chile.*

It takes two to tango: Widening our understanding of the origin of schizophrenia from a neurovascular perspective
Verónica Palma • *Universidad de Chile, Chile.*

S4 - THE INTERSECTION OF METABOLIC AND INFLAMMATORY MECHANISMS UNDERLYING CARDIOVASCULAR DISEASE; EMERGING EVIDENCE OF SEX DIFFERENCES

Chairs: Patricia Molina (Louisiana State University Health Sciences Center, USA), Heddwen Brooks (Tulane University School of Medicine, USA)

Central Nervous System regulation of metabolism
Andrea Zsombok • *Department of Physiology, Tulane University School of Medicine, USA.*

Immuno-metabolic mechanisms of alcohol-associated metabolic instability and their contribution to aging comorbidities
Patricia Molina • *Louisiana State University Health Sciences Center, USA*.

Sex differences in vascular inflammation
Justin P. Van Beusecum • *Department of Medicine, Division of Nephrology Medical University of South Carolina Charleston, USA.*

S5 - CROSS TALK BETWEEN DIFFERENT ORGANS

Chairs: Pedro Leme (Federal University of Rio de Janeiro, Brazil), Marcio Moraes (Federal University of Minas Gerais, Brazil)

Cross talk between lung and brain
Pedro Leme Silva • *Federal University of Rio de Janeiro, Brazil*.

Cross talk between brain and heart
Marcio Moraes • *Federal University of Minas Gerais, Brazil.*

Cross talk between kidney and other organs
Niels Olsen Saraiva Camara • *São Paulo University, Brazil.*

S6 - NEW HORIZONS IN CARDIORENAL ION TRANSPORT

Chairs: Oleg Palygin (Medical University of South Carolina, USA), Daria Ilatovskaya (Augusta University, Medical College of Georgia, USA)

Mitochondrial calcium uniporter complex and its physiological and pathological roles in the heart
Jin O-Uchi • *Department of Medicine, Cardiovascular Division, University of Minnesota, USA*.

Ghrelin enhances tubular magnesium absorption in the kidney
Matthias Wolf • *Department of Pediatrics, UT Southwestern and Children's Medical Center Dallas, USA.*

Water is life: defending against dehydration
Aylin Rodan • *The University of Utah, Internal Medicine, Salt Lake City, USA.*
Protease activated receptors and glomerular function
Oleg Palygin • *Medical University of South Carolina, USA.*

S7 - NOVEL ASPECTS OF CELL COMMUNICATION IN THE MICROCIRCULATION

Session in Honor of Professor Walter Durán
Chairs: Mauricio Boric (Pontificia Universidad Católica de Chile, Chile), Daniel González-Reinoso (Universidad de Talca, Chile)

Limiting Microvascular Hyperpermeability in the Injured Host
Jerome W Breslin • *Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida, Tampa, Florida, USA.*

eNOS signaling via S-nitrosylation in leukocyte and tumor cell adhesion
Fabiola Sánchez • *Universidad Austral de Chile, Chile.*

Connexins and pannexins in the regulation of vascular tone
Xavier Figueroa • *Pontificia Universidad Católica de Chile, Chile.*

Potential use of circulating microalgae for photosynthetic tissue oxygenation. Systemic and ex vivo approaches
Mauricio P Boric • *Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Chile.*

S8 (Sponsored by ELSEVIER) - LIPID METABOLISM AND ADIPOSE TISSUE IN EATING BEHAVIOR AND METABOLIC REGULATION

Chairs: René Braudand (Pontificia Universidad Católica de Chile, Chile), José Galgani (Pontificia Universidad Católica de Chile, Chile)

Risk factors for adiposopathy across the lifespan
Jennifer Thompson • *Department of Biochemistry and Molecular Biology, University of Calgary, Canada.*

Determinants of insulin resistance-associated fatty liver disease
Víctor Cortés • *Pontificia Universidad Católica de Chile, Chile.*

Diet induces neural plasticity-associated modifications and epigenetic changes in the hypothalamus
Bredford Kerr • *Universidad San Sebastián, Chile.*

Insights into feeding behaviour in different environments: From animal models to humans
Claudio Pérez • *Pontificia Universidad Católica de Chile, Chile.*

S9 - HOT TOPICS IN CHRONIC KIDNEY DISEASE

Chairs: Timo Rieg (University of South Florida, USA), Jessica Dominguez (University of South Florida, USA)

Intracellular calcium signalling in podocytes in diabetic nephropathy
Alexander Staruschenko • *University of South Florida, USA*.

The role of Atrial Natriuretic Peptide signalling in kidney disease progression
Daria Ilatovskaya • *Department of Physiology Medical College of Georgia, Augusta University, USA.*

The development and application of a nanoparticle delivery system that selectively targets kidney glomeruli
Ruisheng Liu • *Department of Molecular Pharmacology and Physiology, University of South Florida, USA.*

Iron deficiency anaemia – microbiome changes in response to intravenous iron administration
Jessica Dominguez • *Department of Molecular Pharmacology and Physiology, University of South Florida, USA.*

S10 - PHYSIOLOGICAL AND MOLECULAR REGULATION OF THE HYPOTHALAMIC-NEUROHYPOPHYSIAL SYSTEM

Chairs: André Mecawi (Federal University of São Paulo, Brazil), David Murphy (University of Bristol, UK)

Multi-omics analyses of the hypothalamic-neurohypophysial system
André Mecawi • *Department of Biophysics, Federal University of São Paulo - UNIFESP, Brazil.*

Astrocytic modulation of the hypothalamic magnocellular neurons activity in the supraoptic nucleus
Melina Pires da Silva • *Department of Biophysics, Federal University of São Paulo - UNIFESP, Brazil.*

Sex differences in the neurohypophyseal system in an animal model of cirrhosis
Thomas Cunningham • *Department of Physiology and Anatomy, University of North Texas Health Science Center at Fort Worth, USA.*

Effect of early programming stimuli on magnocellular neurons and their osmoregulatory responses
Andrea Godino • *The Medical Research Institute Mercedes and Martín Ferreyra, INIMEC-CONICET/Faculty of Psychology, National University of Córdoba, Argentina.*

S11 - MUSCLE-ORGAN CROSSTALK: FOCUS ON DISEASES

Chairs: Paola Llanos (Universidad de Chile, Chile), Denisse Valladares (Universidad de O´Higgins, Chile)

Molecular linkers between skeletal muscle atrophy and bone loss after muscle paralysis
Sonja Buvinic • *Universidad de Chile, Chile.*

Gut-Muscle-Brain axis: The role of gut microbiota on muscle and cognitive function during old age
Gonzalo Jorquera • *INTA, Universidad de Chile, Chile*.

Exercise regulation of hepatic LD-mitochondria interaction in non-alcoholic fatty liver disease
Rodrigo Troncoso • *INTA, Universidad de Chile, Chile*.

Connecting the dots: how muscle-resident fibro-adipogenic precursors and adipocytes support muscle tissue metabolic flexibility
Sebastian Jannas Vela • *Universidad de O'Higgins, Chile*.

S12 - HEART FAILURE: MORE THAN A CARDIAC DISEASE

Chairs: Luciana Venturini Rossoni (Brazil), Gerardo García Rivas (Tecnológico de Monterrey, México)

Mechanisms underlying the cardiorenal benefits of SGLT2 inhibitors in heart failure
Adriana Castello Costa Girardi • *Medical School, University of Sao Paulo, Brazil.*

The excitation-contraction coupling is profoundly altered in ventricular cardiomyocytes of a novel model of heart failure with preserved ejection fraction
Gerardo García Rivas • *The Institute for Obesity Research, Tecnológico de Monterrey, Centro de Investigación Biomédica, Hospital Zambrano Hellion, TecSalud, San Pedro Garza García, México.*

Vascular dysfunction in heart failure
Luciana Venturini Rossoni • *Physiology and Biophysics Department, Biomedical Science Institute, University of Sao Paulo, Brazil.*

Brazilian longitudinal study of adult health (ELSA-Brazil): What are we learning?
José Geraldo Mill • *Department of Physiological Sciences, Federal University of Espirito Santo, Brazil.*

S13 - NEW PHYSIOLOGICAL AND THERAPEUTIC FRONTIERS OF THE INTRA-RENAL RENIN ANGIOTENSIN SYSTEM

Chair: Alexis A González (Pontificia Universidad Católica de Valparaíso, Chile)

Non-canonical pathways for renin regulation in the kidney
Lucienne Morcillo • *Universidade Federal do Rio de Janeiro, Brazil.*

Antibody-based detection of Angiotensin receptors in the kidney. Challenges and implications in studying regulatory mechanisms
Marcela Herrera • *Labidech Análisis Clínicos, Vita Medicina Reproductiva, Chubut, Argentina.*

The prorenin receptor (PRR) in physiology and its impact on hypertension and diabetes
Minolfa C Prieto • *Department of Physiology, Tulane University, USA.*

Metabolic pathways involved in the regulation of the (pro)renin receptor in the renal collecting duct
Alexis A González • *Pontificia Universidad Católica de Valparaíso, Chile.*

S14 - MINING THE WAVEFORM, NEW APPROACHES TO DELINEATING RESPIRATORY FEATURES AND ANALYZING RESPIRATORY OUTCOMES

Chair: Russell Ray (Baylor College of Medicine, USA)

A cognitive framework for applying machine learning to neurophysiological assays
Jose Otero • *Department of Pathology, The Ohio State University College of Medicine, USA.*

Quantification of non-linear variability in cardiorespiratory control: Open-source tools for physiology signal analysis
Christopher Wilson • *Departments of Pediatrics and Basic Sciences (Division of Physiology), Loma Linda University, USA.*

Automated interrogation of waveforms designed for respiratory waveform analysis
Savannah Lusk • *Department of Neuroscience, Baylor College of Medicine, USA.*

Analysing polysomnography traces
Jan-Marino Ramírez • *University of Washington School of Medicine, USA.*

S15 (Sponsored by ELSEVIER) - OBESITY AND THE RISK OF CARDIOMETABOLIC DISEASES

Chairs: Gerardo García-Rivas (Tecnológico de Monterrey, México), Marco Rito-Palomares (Tecnológico de Monterrey, México)

Previous cardiovascular injury is a prerequisite for immune checkpoint inhibitor-associated lethal myocarditis. A preclinical study in hypertensive mice
Elena Cristina Castillo • *Tecnológico de Monterrey, Mexico.*

Fatty liver disease and cardiac dysfunction: perspectives from a preclinical study of heart failure with preserved ejection fraction
Omar Lozano • *Tecnológico de Monterrey, Mexico.*

Changes in [glucose]e induce cardiac ventricular arrhythmias partially dependent on SGLT1 activity in mice
Julieta Palomeque • *Universidad de La Plata, Argentina.*

S16 - RECENT ADVANCES IN THE PHYSIOLOGY OF THE IMMUNE SYSTEM IN HEALTH AND DISEASE

Chair: Paola Murgas Alcaíno (Chilean Society of Immunology, SOCHIM)

The cGAS/STING signalling pathway: Its contribution to the inflammaging process
Paola Murgas Alcaíno • *Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Chile.*

Beyond the central dogma: exploring the world of LncRNAs and their impact on immunity against bacterial pathogens
Manuel Flores • *Universidad de Concepción, Chile.*

Equine asthma: A model of neutrophilic phenotype asthma in humans
Gabriel Morán • *Universidad Austral de Chile, Chile.*

S17 - PEPTIDE MODULATION IN SYSTEMS PHYSIOLOGY

A symposium sponsored by the International Regulatory Peptide Society, affiliated to IUPS
Chairs: Limei Zhang (Universidad Nacional Autónoma de México, Mexico), Valery Grinevich (Heidelberg University, Germany)

Oxytocin protects nigrostriatal dopamine system in Parkinson's disease model
Lei Xiao • *The State Key Laboratory of Medical Neurobiology, MOE Frontiers Center for Brain Science and the Institutes of Brain Science Fudan University, China.*

Pituitary adenylate cyclase-activating polypeptide (PACAP) neurocircuitry for endocrine and behavioral stress responses
Sunny Jiang • *Section on Molecular Neuroscience & Dendritic Dynamics Hub, National Institute of Mental Health Intramural Research Program, NIH, USA.*

Novel neuropeptide roles in synaptic structure and synaptic pruning in hippocampus: effects of vasopressin on PSD proteins and microglial activity
Limei Zhang • *Physiology, Medicine, Universidad Nacional Autónoma de México, México.*

Using a novel transgenic AVP-Cre rat to dissect vasopressin circuits in the brain and their behavioral roles
Quirin Krabichler • *Central Institute of Mental Health (CIMH-ZI), Department of Neuropeptide Research in Psychiatry, School of Medicine, Heidelberg University, Germany.*

S18 - CONNECTING STUDENTS WITH THE COMMUNITY TO ENHANCE LEARNING

Chairs: Patricia A. Halpin (University of New Hampshire, USA), Victoria Velarde (Universidad de Valparaíso, Chile)

Using a role play activity with life science and American Sign Language (ASL) interpreting students to provide undergraduates experience in the healthcare setting
Patricia A. Halpin • *University of New Hampshire, Department of Life Sciences, Manchester NH, USA.*

Reflective diaries as a tool for the development of metacognition in students from a physiology course
Loreto Véliz • *Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Chile.*
Carolina Serrano • *Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Chile.*
Victoria Velarde • *Faculty of Sciences, Universidad de Valparaíso, Chile.*

IUPS efforts stimulate broader community engagement
Robert Carroll • *Brody School of Medicine, East Carolina University, USA.*

S19 - RECENT ADVANCES AND FUTURE AVENUES IN UNDERSTANDING OBESITY AS A PREMATURE AGING PHENOTYPE

Chairs: María Paulina Correa (INTA, Universidad de Chile, Chile), Christian González-Billault (Universidad de Chile, Chile)

NOTE: *S19 Cancelled by the Chairs (Nov 14, 2023)*

S20 - NEW PATHOLOGICAL MECHANISMS OF CARDIOVASCULAR DISEASES

Chairs: Sergio Lavandero (Universidad de Chile, Chile), Mario Chiong (Universidad de Chile, Chile)

Cardioprotection by endothelial small extracellular vesicles
Jaime Riquelme • *Advanced Center for Chronic Diseases, Universidad de Chile, Chile.*

Role of the mitochondrial protease Clpp in the vasculature
Alejandra San Martín • *Universidad Andrés Bello, Chile.*

Primary cilia in cardiac fibrosis
Sergio Lavandero • *Advanced Center for Chronic Diseases, Universidad de Chile & UT Southwestern Medical Center Dallas, USA.*

New insight of the renin angiotensin system on vascular remodelling
Mario Chiong • *Advanced Center for Chronic Diseases, Universidad de Chile, Chile.*

S21 - NEW INSIGHTS INTO THE STUDY OF ADAPTIVE AND MALADAPTIVE MYOCARDIAL GROWTH

ISHR LAT symposium
Chairs: Martín Vila Petroff (CONICET-UNLP, Argentina), Irene L. Ennis (CONICET-UNLP, Argentina)

Regulation of mitochondrial function and morphology during cardiomyocyte adaptative growth
Sergio Lavandero • *Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemistry and Pharmaceutic Sciences, Faculty of Medicine, Universidad de Chile, Chile & University of Texas Southwestern Medical Center Dallas, USA.*

Role of the alkalinizing transporters in the development of pathological cardiac hypertrophy
Alejandro Aiello • *Centro de Investigaciones Cardiovasculares "Horacio E. Cingolani", Faculty of Medical Sciences, CONICET-Universidad Nacional de La Plata, Argentina.*

Mitochondrial dysfunction in cardiac hypertrophy and failure: chicken or egg? Judith Bernal Ramírez • *Tecnológico de Monterrey, The Institute for Obesity Research, Hospital Zambrano Hellion, San Pedro Garza García, México.*

Apelin signalling pathway as a mediator of cardioprotection in the hypertrophied myocardium
Alejandra M. Yeves • *Centro de Investigaciones Cardiovasculares "Horacio E. Cingolani", Faculty of Medical Sciences, CONICET-Universidad Nacional de La Plata, Argentina.*

S22 - NOCICEPTION AND PAIN: FROM MECHANISMS TO THERAPEUTIC APPROACHES

Chairs: Trinidad Mariqueo (Universidad de Talca, Chile), Carolina A Oliva (Universidad Autónoma de Chile)

Molecular and cellular elements of thermal sensitivity in thermoTRP channels and their modulation as a therapeutic target for pain relief
Karen Castillo • *Centro de investigación de Estudios Avanzados del Maule, Vicerrectoría de Investigación y Postgrado Universidad Católica del Maule, Talca, Chile. Centro Interdisciplinario de Neurociencia de Valparaíso, Instituto de Neurociencia, Universidad de Valparaíso, Valparaíso, Chile.*

The role of inhibitory currents in sex-dependent pain perception processing in the central amygdala
Carolina A Oliva • *Centro para la Transversalización de Género en I+D+i+e, Universidad Autónoma de Chile.*

Animal models to measure itching, acute and chronic pain, and their use for novel drug development
Jimmy Stehberg • *Universidad Nacional Andrés Bello, Chile.*

The role of inhibitory glycinergic synapsis of central amygdala in the neuroimmune modulation of chronic pain
Trinidad Mariqueo • *Universidad de Talca, Chile.*

S23 - PATHOPHYSIOLOGY SIGNALLING MECHANISMS IN DISEASES

Chair: Daniel Peluffo (Universidad de la República, Uruguay)

Endothelial Connexin 43 hemichannels a key relaxation vascular component in female breeder mice
Mauricio Lillo • *Department of Pharmacology, Physiology & Neuroscience, Rutgers - New Jersey Medical School, Rutgers, The State University of New Jersey, USA.*

Role of NOX2 in the dystrophic cardiomyopathy
Daniel González-Reinoso • *Faculty of Health Sciences, Universidad de Talca, Chile.*

Modulation of L-arginine transport by nitric oxide: pathophysiological implications
Daniel Peluffo • *Group of Biophysical Chemistry, Department of Biological Sciences, CENUR Litoral Norte - sede Salto, Universidad de la República, Uruguay.*

Adenosine/L-arginine-NO signalling in human placenta endothelium from gestational diabetes mellitus Luis Sobrevia • *Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Faculty of Medicine, Pontificia Universidad Católica de Chile, Chile.*

S24 - ROLE OF THE IMMUNE SYSTEM IN HYPERTENSION AND DIABETES

Chairs: Luis Michea (Universidad de Chile, Chile), Kristine Deleon-Pennell (Medical University of South Carolina, USA)

Sex differences in T cell mediated hypertension
Heddwen Brooks • *Department of Physiology, Tulane University School of Medicine, New Orleans, USA.*

CD8+ T-cells negatively regulate cardiac tissue biomechanics post-MI
Kristine DeLeon-Pennell • *Medical University of South Carolina, USA.*

Antigen-presenting cell modulation of blood pressure
Luis Michea • *ICBM-Hospital Clínico Universidad de Chile, Faculty of Medicine, Universidad de Chile, Chile.*

Neutrophil gelatinase-associated lipocalin as an immunomodulator in hypertension USS
Cristian Amador • *Faculty of Medicine and Science, Universidad San Sebastián, Chile.*

S25 - HORMONAL SIGNALING IN CARDIOVASCULAR DISEASE

ISHR LAT symposium
Chairs: Celeste Villa-Abrille (CONICET-UNLP, Argentina), Zully Pedrozo (Universidad de Chile, Chile)

Adrenocortical hormones and cardiac dysfunction
Gustavo Pérez • *Centro de Investigaciones Cardiovasculares "Horacio Cingolani", Faculty of Medical Sciences, CONICET-Universidad Nacional de La Plata, Argentina.*

Oestrogen signalling as a bridge between the nucleus and mitochondria in cardiovascular diseases
Valentina Parra • *Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemistry and Pharmaceutic Sciences, Faculty of Medicine, Universidad de Chile, Chile.*

Cardiac dysfunctional hormonal signalling during menopause
Valeria Martínez • *Centro de Investigaciones Cardiovasculares "Horacio Cingolani", Faculty of Medical Sciences, CONICET-Universidad Nacional de La Plata, Argentina.*

S26 - HORMONES AND CONTROL OF BREATHING

Chairs: Vsevolod Polotsky (George Washington University, USA)

Melanocortins: new players or forgotten players in control of breathing?

Vsevolod Polotsky • *Department of Anesthesiology and Critical Care Medicine, George Washington University, USA.*
Sex hormones and control of breathing
Luciane Gargaglioni • *Department of Animal Morphology and Physiology, Faculty of Agriculture and Veterinary Sciences, UNESP, Brazil*.

Stress, orexin, and control of breathing in female rats: insights in the pathophysiology of panic disorders
Richard Kinkead • *Department of Pediatrics, University of Laval, Canada.*

Oxytocin and control of breathing
David Mendelowitz • *Department of Pharmacology and Physiology, George Washington University, USA).*

S27 - VASCULAR DYSFUNCTION WITH COVID-19

Chairs: Shampa Chatterjee (University of Pennsylvania School of Medicine, USA), Amaro Nunes Duarte Neto (Universidade de São Paulo, Brazil)

Vascular Transformation from COVID-19 to Long-COVID
Douglas Fraser • *Western University, London, Canada*.

Endothelial oxidant signalling post SARS-CoV-2 infection
Shampa Chatterjee • *University of Pennsylvania School of Medicine, Philadelphia, USA.*

Ultrastructural findings in fatal COVID-19: Vessel injury and endothelialitis
Amaro Nunes Duarte Neto • *Universidade de São Paulo, Brazil.*

S28 - MOLECULAR AND PHYSIOLOGICAL MECHANISMS OF THE EARLY PROGRAMMING OF DISEASES

Chair: Paola Casanello (Pontificia Universidad Católica de Chile, Chile)

Adolescence: Last chance to re-programming health or disease
Paulo Cezar de Freitas Mathias • *Universidade Estadual do Maringá, Maringá, Brasil.*

Gestational hypothyroxinemia impact offspring health
Claudia Riedel • *Universidad Andrés Bello, Santiago, Chile.*

Peri-gestational intake of excess added sugars as a risk factor for the early onset of NAFLD in the offspring
Antonio Marcus de Andrade Paes • *Universidade Federal do Maranhão, Maranhão, Brazil.*
Early programming of obesity in the offspring of women with gestational obesity: insights into mechanisms and opportunity for interventions
Paola Casanello • *Pontificia Universidad Católica de Chile, Santiago, Chile.*

S29 - CHANNELS AND MEMBRANE TRANSPORT IN DISEASES

Chair: Gonzalo Ferreira (Universidad de La República, Uruguay), Luis Sobrevia (Pontificia Universidad Católica de Chile, Chile)

Bacterial toxins and heart function: heat labile Escherichia coli enterotoxin B promotes changes in cardiac function with possible relevance for sudden cardiac death

Gonzalo Ferreira • *Department of Biophysics. School of Medicine, Universidad de La República, Montevideo. Uruguay.*
Role of RyR2 phosphorylation in myocardial stunning/infarction, cardiac arrhythmias and calcium alternans
Carlos Valverde • *Centro de Investigaciones Cardiovasculares 'Dr. Horacio E. Cingolani', Faculty of Medical Sciences, UNLP/CCT-CONICET, Argentina.*

Connexin channels as mediators of cardiac stress-induced arrhythmias and myocardial infarction
Jorge Contreras • *Department of Physiology and Membrane Biology, School of Medicine, University of California Davis, USA*.

Cellular and molecular mechanisms of mammalian proprioception
Theanne Griffith • *Department of Physiology and Membrane Biology, School of Medicine, University of California Davis, USA.*

S30 - CHEMORECEPTORS IN HEALTH AND DISEASE: EXPLORING NEW AVENUES OF TREATMENT

Chairs: Camilo Toledo (Universidad Austral de Chile, Chile)

Peripheral chemoreception and the control of breathing
Rodrigo Iturriaga • *Department of Physiology, Universidad de Antofagasta, Chile.*

Central chemoreception and breathing control
Jaime Eugenin • *Departamento de Biología, Universidad de Santiago de Chile, Chile.*

Altered breathing control in diabetes and the role of chemoreception
Silvia Conde • *NOVA Medical School, Universidade Nova de Lisboa, Portugal.*

Peripheral chemoreceptors and regulation of kidney function in sleep apnea and heart failure
Noah Marcus • *Department of Physiology and Pharmacology, Des Moines University, Des Moines, USA.*

S31 - MYOFILAMENT-BASED MECHANISMS OF MUSCLE DISEASE

Chair: Henk Granzier (University of Arizona, USA)

Modulation of cardiac function by cardiac myosin light chain phosphorylation
Audrey Chang • *University of Texas Southwestern Medical Center, Dallas, USA.*

Roles of myosin-binding protein C in cardiac contraction, disease and therapy
Brett Colson • *University of Arizona, Tucson, USA.*

Myosin light chain mutant induced cardiomyopathies
Danuta Szczesna-Cordary • *University of Miami, Miller School of Medicine, Miami, USA.*

Structure-function relationship of thin filament regulatory protein in cardiovascular health
J. P-Jin • *University of Illinois at Chicago, College of Medicine, USA.*

ORAL COMMUNICATIONS

OC1 CARDIOVASCULAR AND RESPIRATORY

Chair: Pedro Leme Silva (Federal University of Rio de Janeiro, Brazil)

Post-traumatic stress disorder (PTSD) induces a greater negative effect on the cardiovascular system in females compared to males
Kristine DeLeon-Pennell • *Medical University of South Carolina, USA*.

Role of angiotensin converting enzyme 2 in the cardioprotective effect of endothelial small extracellular vesicles
Constanza Rimassa Taré • *Universidad de Chile, Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas & Farmacéuticas y Facultad de Medicina, Chile.*

Obligatory and accessory muscle contribution to peak inspiratory performance in age-related sarcopenia
Ben Murphy • *University College Cork, Department of Physiology, School of Medicine, College of Medicine and Health, Ireland.*

Inhibition of mitochondrial protein kinase D protects right ventricles from cardiac fibrosis and dysfunction under pulmonary arterial hypertension
Bong Sook Jhun • *University of Minnesota, Department of Medicine, Cardiovascular Division, Lillehei Heart Institute, Minneapolis, USA.*

OC2 RENAL AND GASTROINTESTINAL

Chair: María Cecilia Larocca (Institute of Experimental Physiology of Rosario, IFISE-CONICET-UNR, Argentina)

Enhancing renal graft function and alleviating ischemic kidney injury through synchronized modulation electric field for Na^+/K^+-ATPase maintenance
Lei Wang • *University of South Florida, Molecular Pharmacology and Physiology, Tampa, USA.*

Mechanistic insight into angiotensin II type 2 receptor (AT2R) nephroprotective effect during renal ischemia/reperfusion
Tomas Maknis • *Instituto de Fisiología Experimental (IFISE-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (FBIOyF-UNR), Argentina.*

Galectin-8 counteracts acute kidney injury induced by folic acid
Elisa Pérez-Moreno • *Centro de Biología Celular y Biomedicina (CEBICEM), Facultad de Medicina y Ciencia, Universidad San Sebastián & Centro Científico y Tecnológico de Excelencia (CCTE) Ciencia & Vida, Chile.*

NBCe1 KO mice exhibit changes in the mRNA expression of SCL4 family transporters in gastrointestinal system
Carlos Spichiger • *Universidad Austral de Chile, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Chile.*

OC3 SKELETAL MUSCLE AND EXERCISE PHYSIOLOGY

Chair: Sonja Buvinic (Universidad de Chile, Chile)

The structure, stability, and function of the neuromuscular synapse is altered upon activation of the canonical Wnt signalling pathway
Juan Pablo Henríquez • *Universidad Austral de Chile, Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Valdivia, Chile.*

CCL5/RANTES induces a sarcopenic phenotype through a mechanism dependent on NADPH oxidase and NF-kB
Francisco Aguirre Galaz • *Department of Biological Sciences, Faculty of Life Sciences & Millennium Institute of Immunology and Immunotherapy, Faculty of Life Sciences, Andrés Bello University, Chile.*

Fecal microbiota transplant from young-trained donors reduces intestinal permeability in old mice
Constanza Cáceres • *Centro de Neurobiología y Fisiopatología Integrativa (CENFI), Departamento de Fisiología, Facultad de Ciencias, Universidad de Valparaíso, Chile.*

Moderate-intensity constant and high-intensity interval training confer differential metabolic benefits in skeletal muscle, white adipose tissue, and liver of candidates to undergo bariatric surgery
Sergio Martínez-Huenchullán • *Universidad Austral de Chile, Chile.*

OC4 LIPIDS AND NUTRITION

Chair: Paola Llanos (Universidad de Chile, Chile)

Docosahexaenoic acid (DHA) supplementation during pregnancy increases the expression of placental antioxidant enzymes in pregnant women with gestational diabetes mellitus
Karina Etchegaray • *Institute of Nutrition and Food Technology- INTA, Universidad de Chile & School of Nutrition and Dietetics, Faculty of Medicine, Finis Terrae University, Chile.*

Deleterious effects of high-fructose diets in BALB/c mice
Lorena Mardones • *Universidad Católica de la Santísima Concepción, Research Laboratory in Biomedical Sciences, School of Medicine & Research Center on Biodiversity and Sustainable Environments, Concepcion, Chile. Epidemiology of Lifestyle and Health Outcomes in Chile Consortium, Chile.*

Acquired long QT syndrome caused by hERG Potassium channel block in isolated hearts of Cavia porcellus (guinea pig) and its reversion by membrane lipid replacement with nanomicelles
Luisina Chavarría • *Universidad de la República, Departamento de Biofísica, Facultad de Medicina, Montevideo, Uruguay.*

Sex-modulated transcriptomic changes in the interaction between leptin, GLP-1, and glucocorticoid receptors in the postprandial response to chronic high-fat diet
Yanireth Jimenez • *Pontificia Universidad Católica de Chile, Faculty of Biological Sciences, Chile.*

OC5 IMMUNOLOGY AND CANCER

Chair: Liliana Lamperti (Universidad de Concepción, Chile)

Modulation of the immune response by a bacterial consortium applied in mice infected with Salmonella typhimurium
Diana Lucía Díaz-Montoya • *Catholic University of Santa Maria, Department of Human Medicine, Faculty of Human Medicine & National University of San Agustin, Biology, Biological Sciences, Arequipa, Perú.*

Protective effect of alamandine in a melanoma solid tumour and cancer associated cachexia model
Filipe Alex Silva • *Federal University of Minas Gerais, Physiology and Biophysics, Institute of Biological Sciences, Belo Horizonte, Brazil.*

Cytokine profiling in workers at high altitude subjected to chronic intermittent hypoxia
Rodrigo Calderón Jofré • *Universidad Católica del Norte, Departamento de Ciencias Biomédicas, Facultad de Medicina, Coquimbo, Chile.*

Changes on microglia-neuron interaction in aging and neuroinflammation
Rommy von Bernhardi • *Universidad San Sebastian, Health Care Sciences, Santiago, Chile.*

OC6 ENDOCRINE AND METABOLISM

Chair: Carlos Escudero (Universidad del Bio Bio, Chile)

Human adipose-derived extracellular vesicles (AdEVs) increase proinflammatory markers in renal and endothelial cells: a preliminary study
Cristian Carvajal • *Pontificia Universidad Católica de Chile, Endocrinology, Faculty of Medicine & CETREN, Santiago, Chile.*

Andro-mediated modulation of glucose metabolism in visceral adipose tissue in Alzheimer's disease model
Evrim Servili • *Universidad de O'Higgins, Instituto de Ciencias de la Salud, Rancagua, Chile.*

Human umbilical vein endothelial cells dysfunction in gestational diabesity
Paola Valero • *Universidad de Talca, Faculty of Health Sciences, Talca, Chile & Pontificia Universidad Católica de Chile, Medical Research Center, Santiago, Chile.*

High-fat/low-carb diet induces neural plasticity-associated modifications and changes in epigenetic factors in the arcuate nucleus of the hypothalamus
Cristina Silva • *Universidad San Sebastián, Centro de Biología Celular y Biomedicina, Medicina y Ciencias, Santiago, Chile.*

OC7 EDUCATION

Chair: Dee Silverthorn, (University of Texas, USA)

Development of a thermostatic temperature control system to teach concepts on physiological regulation

Paulo Montenegro • *Federal University of Paraiba, Systematics and Ecology Department, Center for Exact and Natural Sciences, PB, Brazil.*

Effect of blended teaching associated with formative assessments on learning about control of blood pressure and on pre-test stress and anxiety
Fernanda Klein Marcondes • *University of Campinas, Department of Biosciences, Piracicaba Dental School, Brazil & State University of São Paulo, Education, Study and Research Group, University Pedagogy, Institute of Biosciences, Rio Claro, Brazil.*

The identification of behavioral patterns seems to improve e-learning of integrative physiology through core concepts
Sánchez-Briones • *Universidad Autónoma de San Luis Potosí, Facultad de Estudios Profesionales Zona Huasteca, San Luis Potosí, México.*

Investigating the human physiology through smartphone-assisted experimentation: development of a rubric and outcomes of pre-service science teachers
Camilo Lellis-Santos • *Universidade Federal de São Paulo, Biological Sciences, Institute of Environmental, Chemical and Pharmaceutical Sciences, Diadema, Brasil.*

OC8 NEUROPHYSIOLOGY

Chair: Bredford Kerr (Universidad San Sebastián, Chile)

The astrocytes from the supraoptic nucleus are activated by hypertonic solution and contribute to magnocellular depolarization
Karoline Martins dos Santos • *Federal University of Sao Paulo, Department of Biophysics, Paulista School of Medicine, Sao Paulo, Brazil*.

Functional role of the K^+ channel Kir7.1 in the transepithelial transport of choroid plexuses
Juan Carlos Henao Barbsa • *Centro de Estudios Cientificos (CECs) & Universidad Austral de Chile, CIencias, Valdivia, Chile.*

The extracellular matrix protein osteopontin regulates aquaporin-4 expression by activating the calcium channel TRPV4 in retinal Müller cells
Vanina Netti • *Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Argentina.*

Neuroendocrine, metabolic and locomotor adaptations in the L-NAME model of preeclampsia in mice
Gustavo Moreira • *Federal University of São Paulo, Biophysics, Paulista School of Medicine, São Paulo, Brazil.*

WORKSHOPS

W1 - WORKSHOP PHYSIOLOGY AND NARRATIVES

Coordinator: Wilson Andrés Parra Chico (Colombia)
Speakers: Leonardo Gómez Duarte (Veterinarian) • *Universidad Nacional de Colombia.*
Iris del Mar Lineros (Physiotherapist) • *Universidad Nacional de Colombia.*
Wilson Andrés Parra Chico (MD) • *Universidad de la Sabana, Colombia.*
Carlos Orlando Wilches (Psychologist) • *Unigermana Universidad del País Vasco, Spain.*
Allocated time: 45 min
Topics: Southern physiology and epistemologies
Narrative methodology applied to research in physiology
Physiology and narrative didactics
Description
The transversal question of our work is why using a human sciences approach can be useful in an exact science such as physiology is considered? During the last 10 years, we focused on understanding the identity of the physiologists in Latin America. Through courses in the history of physiology, epistemology of physiology and didactics of physiology, we favored disciplinary reflection to give postgraduate training in science a humanistic character. Currently the group has appropriated a voice in contemporary physiology in the context of (1) narrative inquiry methods applied to physiology, (2) fluid physiology and pertinent in addressing complex problems, and (3) we are a physiology didactics laboratory to the extent that we value the voice and experience of the student who intends to acquire physiological thinking.
The objective of our workshop is to demonstrate the need for the relationship between human sciences and physiology. The narrative approach applied to educational practices has emerged with great force since the late eighties at the head of Anglo-Saxon research groups mainly from Canada and the United States. Narratives have been used with greater emphasis for two purposes, namely 1) to understand how teaching practices have been configured from the story of experiences in life cycles and as an element in the pedagogical knowledge of the contents, and 2) the role of the narrative in the formation of students, mainly in the field of morality. Teaching and learning within educational practices are an imminently social process, which develops from and in the joint experience of all those who share this scenario. It is precisely in those accounts of the experience configured in community where important elements are found to understand what teachers do and at the same time for students to understand what they do as professionals. In the context of Physiology, we consider life as its object of study, and we intend to convey the idea of the physiologist as a cultivator of the living in the Latin American context.

W2 - TISSUEGNOSTICS (Sponsored by URSULAB Chile)

Coordinator: Juan Carlos Torres (URSULAB Chile)
Speakers: Robert Nica • *Tissuegnostics, Austria.*
Henning Ulrich • *Tissuegnostics, Austria.*
Topics: Software and artificial intelligence
TissueFaxs
Whole-slide imaging (brightfield, fluorescence, confocal, multispectral)
High-end analysis of tissue sections
Allocated time: 45 min

W3 - PRECISION IN BREATHING, A WORKSHOP ON SMALL ANIMAL ADULT AND NEONATE PLETHYSMOGRAPHY

Coordinator: Russell Ray (USA)
Speakers: Russell Ray (Associate Professor) • *Department of Neuroscience, Baylor College of Medicine, USA.*
Kevin Cummings (Associate Professor) • *Department of Biomedical Sciences, University of Missouri, USA.*
Topics: Advances in small animal cardio-respiratory measurements
Key fundamentals and best practices
Allocated time: 45 min

Description

The aim of this workshop is to cover the recent advances in small animal cardio-respiratory measurements while revisiting key fundamentals and best practices that have been, at times, overlooked. Respiratory measurements are being increasingly recognized as important outcome measures in a variety of congenital, neurodegenerative, affective and infectious disease models that inform upon disease mechanism and clear a path toward therapeutic and diagnostic advances. Poor execution of these techniques will lead to wasted resources, and erroneous results that misdirect translational efforts. Thus, there is a need in the field to both revisit key fundamentals in breathing studies as well as to highlight novel advances in the field.

Adult respiratory measurement techniques will focus on conscious, unrestrained whole body barometric plethysmography (WPB) in rodents. Turnkey and bespoke systems will be compared and contrasted. Key plethysmographic features needed for effective respiratory measurements will be covered. Caveats, pitfalls, and common mistakes will be discussed. Lastly, new approaches in adult respiratory measurements going beyond WBP will be previewed.

Neonatal respiratory measurement techniques will focus on facemask pneumotachography. Pneumotachography, whole body barometric, and sealed neck collar approaches for neonates will be contrasted and compared. Key aspects of developing and carrying out neonatal respiratory measurement assays will be discussed with a focus on best practices and potential challenges. Innovative approaches to high-throughput measurement assays will be previewed. Modifications to systems that allow the measurement of additional, non-respiratory variables (blood pressure, heart rate, state of vigilance) in both neonatal and adult rodents will be discussed.

W4 - NEURAL ORBIT (THE NEO PROJECT): IMPLEMENTING NEW PHYSIOLOGICAL TECHNOLOGIES TO MOTIVATE NEW GENERATIONS OF PHYSIOLOGISTS

Coordinators: Alain Riveros-Rivera (Colombia/Germany)
Tatiana Mendes (Brazil)

Speakers: Alain Riveros-Rivera (Medical physiologist) • *Pontificia Universidad Javeriana, Colombia, & Center for Space Medicine and Extreme Environments-Charité Berlin, Germany.*
Tatiana Mendes (Biomedical engineer) • *ADI Training Manager Latin America.*

Topics: Shooting Stars: in this experiment, the participants review the speed of different cosmic phenomena and compare them with physiological ones (e.g., pulse wave and the nerve conduction velocity)
Space Spinning Tops: in this experiment, the participants review the rotation axis of different Solar Systems planets and calculate the cardiac axis using ECG signals.
The Cosmic Elevator: in this experiment, the participants review the concept of microgravity and the effect of gravity on physiological parameters such as pulse rate and blood pressure.

Allocated time: 45 min

Description

Keeping alive the flame for physiological research is one of the responsibilities of those of us who currently live in this science. For this reason, implementing simple but attractive pedagogical strategies in middle and high school students should be part of our actions. The truth is that most of the time, our efforts are focused on research centers or universities, leaving out the younger ones. This is likely due to the limitations in infrastructure and human and technical resources that a physiology laboratory demands. The aforementioned is even more marked in Latin American countries with tight educational budgets. In this context, the NEO project emerges for mixing space with physiological sciences to bring advanced technology resources to vulnerable student populations in Bogotá-Colombia. This project aims to reduce the gaps in access to technology among the different social classes, allowing low-income students to work with university-level equipment.

The objective of this workshop is that participants can perform some practices of the NEO project using portable sensors (Lt sensors technology). These physiological experiments will demonstrate the technology's versatility and how simple demonstrations can motivate students to study physiological and space sciences. As a model, these practices could inspire the teachers in attendance to create their experiments with portable sensors, allowing low-cost experiments to be done in or out-side the laboratory.

W5 - BEST PRACTICES FOR PUBLISHING IN THE AMERICAN JOURNAL OF PHYSIOLOGY – RENAL PHYSIOLOGY

Coordinators: Luis Michea (Chile)
Heddwen Brooks (USA)
Speakers: Heddwen Brooks (Editor-in-Chief AJP-Renal) • *Tulane University, USA.*
Alexander Starushchenko (Deputy Editor-in-Chief AJP-Renal) • *University of South Florida, USA.*
Publishing in the American Journals of Physiology - graphical abstracts.
Timo Rieg (Associate Editor AJP-Renal) • University of South Florida, USA.
Publishing in the American Journals of Physiology - Graphs, Figures and ARRIVE Guidelines.
Luis Michea (Associate Editor AJP-Renal) • Universidad de Chile, Chile.
Publishing in American Journal of Physiology journals
Topics: Aspects of publishing in the AJP-Renal Physiology
Allocated time: 45 min
Description
The goal of this workshop will be to demonstrate different aspects of publishing in the American Journal of Physiology – Renal Physiology. The Editor-in-Chief (Dr. Heddwen Brooks, Tulane University, USA), Deputy Editor in Chief (Dr. Alexander Starushchenko, University of South Florida, USA), Associate Editors Dr. Timo Rieg (University of South Florida, USA) and Dr. Luis Michea (Universidad de Chile, Santiago, Chile) will give presentations about different aspects of the publishing process. The session will be chaired by Drs Brooks and Michea. Dr. Brooks will talk about the state of the journal, including statistics on the geographic region of origin of articles, ongoing calls for papers and the early career fellowship. She will expand on the required rigor and reproducibility for the journal. Dr. Starushchenko will present on how to prepare the newly required graphical abstract with the focus on the best visual presentation and summary of results. Dr. Rieg will discuss ways to present graphs and data as part of figures as well as how to write figure legends according to ARRIVE Guidelines. Dr. Michea will talk about the editorial process, how reviewers are selected and how decisions are made.

W6 - LEVERAGING EDUCATIONAL TECHNOLOGY IN PHYSIOLOGY EDUCATION

Coordinator: Diego F. Niño (USA)
Speakers: Diego F. Niño (MD, PhD, Associate Professor) • *Department of Cell Biology & Pharmacology, Herbert Wertheim College of Medicine (HWCOM), Florida International University, USA.*
Pre-recorded video - Stephanie Tadal (PhD, Director) • *Instructional Design & HWCOM, Florida International University, USA.*
Pre-recorded video - Jessica Campusano (BS, Instructional designer) • *HWCOM, Florida International University, USA.*
Pre-recorded video - Catarina Vale (BS, Medical student CO2025) • *HWCOM, Florida International University, USA.*
Topics: Educational Technology tools needed to promote active learning.
Synchronous and asynchronous instructional materials.
Allocated time: 45 min
Description
The workshop proposed has been designed as a faculty development workshop for health sciences educators. The goal is to provide participants with the tools needed to promote active learning in their own instructional environments through an interactive "hands-on" experience. Participants will use free and open-source software designed to create effective and interactive synchronous and asynchronous instructional materials. The workshop format is used to allow adequate level of interaction among participants and facilitators. Ample time will be provided for participants to learn and practice the skills taught.
This workshop is designed to help health sciences educators gain the skills needed to design and develop effective, interactive, and engaging asynchronous instructional materials to support active learning. We seek to promote the adoption of new educational technologies and approaches that can enrich the learning environment for pre-clinical, clinical, and post-graduate learners. Participation in this workshop will help educators to integrate evidence-based educational practices and appropriate instructional technology through a series of hands-on activity.
At the end of the learning experience the participant will be able to: 1. Understand and apply the Technological Pedagogical Content Knowledge (TPACK) framework for designing a learning experience, 2. Identify applications of the TPACK framework in their teaching environment, 3. Define and review the benefits of Active

Learning, 4. Summarize Cognitive Load and Multimedia Learning Theory, 5. Apply the theories of cognitive load and best practices of multimedia design to create instructional materials including interactive learning modules, videos, podcasts, and infographics.
Throughout the workshop, participants will have the opportunity to work collaboratively with their peers from other institutions to facilitate creative and shared problem-solving. Small groups will be used to ensure a high degree of interaction, and facilitators will be available to help with questions and guide discussion.

W7 - GENERATIVE ARTIFICIAL INTELLIGENCE FOR TEACHERS (Sponsored by the National Academy of Sciences of Honduras and the Embassy of Honduras to Chile)

Coordinator: Mario Lanza Santamaría (Honduras)
Speaker: Mario Lanza Santamaría • *National Academy of Sciences of Honduras*.
Allocated time: 45 min
Topics: GAI applicability
GAI for teaching in physiology and medicine

W8 - DIDACTICS OF SCIENCES APPLIED TO THE TEACHING OF PHYSIOLOGICAL SCIENCES

Coordinator: Monica Reinartz Estrada (Colombia)
Speaker: Monica Reinartz Estrada • *Veterinary Physician, Professor of Physiology, Universidad Nacional de Colombia.*
Allocated time: 45 min
Topics: 1. Some successful teaching and learning methodologies of the physiological sciences.
a. Reinartz Student Seminary
b. neurodidactic fable
2. Conceptual change as a possibility of evaluation in physiology courses.

Methodology
There will be a presentation of two physiology teaching methodologies, which have shown successful results, accompanied by a session on conceptual change and its role in evaluation: it will end with a discussion on the subject among teachers from different countries participating in the congress.

W9 - EXPLORING GAMIFIED PEDAGOGICAL APPROACHES FOR NEUROPHYSIOLOGY IN EDUCATIONAL SPACES

Coordinator: Phoenix Plessas-Azurduy (Canada)
Speaker: Phoenix Plessas-Azurduy (Founder of the ThinkSci Outreach Program) • *McGill University, Montreal, Canada.*
Allocated time: 45 min
Topics: Learner-centered pedagogical approaches used to teach neurophysiology
Tools for electrophysiological recordings: The SpikerBox

Description
The ThinkSci Outreach Program is a workshop-based initiative created to immerse high school seniors and college students into the world of neurophysiology. With a design-thinking approach, gamified to cultivate critical-thinking and problem-solving skills, students get to step into the shoes of neurophysiologists.
We are a team of undergraduate and graduate students who are passionate about physiology and teaching with the aim of supplying opportunities to underrepresented students to experience neurophysiology and empower these students to pursue physiology at the undergraduate and graduate levels/higher education.
Our workshop at PANAM is geared towards educators and investigators alike within the physiology community who are interested in neurophysiology and pedagogy. Here is an overview:
1. A brief introduction to the ThinkSci Outreach Program (our mission and goals)
a. An overview of the structure of our workshops
b. An introduction to the tools we use and pedagogical approaches we employ. Importantly, the SpikerBox: an affordable and reliable electrophysiological recording tool we use at ThinkSci not only to study neurophysiology at the graduate level but also to teach neurophysiology to a wide variety of ages and educational levels.
2. The bulk of this workshop will be a walk-through of our ThinkSci workshops giving educators the chance to experience our workshops from a learner's perspective
a. During this portion, attendees will be split into small-collaborative working groups to importantly allow them to experiment with electrophysiological recording (as students would do in workshop)

b. Additionally, we will periodically interrupt this portion to provide information regarding the pedagogical interventions we choose to employ at ThinkSci to highlight their importance and versatility in application to attendees.
3. To conclude, we will recap salient points and have a Q&A period for attendees to ask any questions they may have.
At the end, attendees will be provided instructions to build their own Spikerboxes in case they would like to order the parts and build them on their own. By the end of our workshop, we hope attendees feel prepared and empowered to bring these tools and approaches to their scientific and educational spaces.

W10 - SCIENTIFIC COMPETITION SESSION IN PHYSIOLOGICAL SCIENCE FOR UNDERGRADUATE STUDENTS PANAM 2023

Coordinator: Ivanita Stefanon (Universidade Federal do Espirito Santo, Vitória, Brazil)
Speakers: Selected from applicants attending PANAM 2023
Topic: Various (depending on selected presentations)
Allocated time: 45 min
Description (more information below for details)
The objective of the Scientific Competition Session in Physiological Sciences is to provide undergraduate students with a platform to showcase their research work and foster academic excellence in the field of physiology. The competition aims to promote scientific inquiry, critical thinking, and effective communication skills among participants. By encouraging active participation and recognizing outstanding achievements, the competition seeks to inspire a passion for physiology research and pave the way for future advancements in the field. Through the presentation, students will have the opportunity to share their findings, methodologies, and conclusions with a panel of evaluators, fostering collaboration and intellectual growth. At the end of the competition, the top three participants will be awarded honorable mentions, acknowledging their exceptional contributions to the field of physiology.

REGULATIONS
1. Objective
The objective of the Scientific Competition Session in Physiological Sciences is to provide undergraduate students with a platform to showcase their research work and foster academic excellence in the field of physiology. The competition aims to promote scientific inquiry, critical thinking, and effective communication skills among participants. By encouraging active participation and recognizing outstanding achievements, the competition seeks to inspire a passion for physiology research and pave the way for future advancements in the field. Through the presentation, students will have the opportunity to share their findings, methodologies, and conclusions with a panel of evaluators, fostering collaboration and intellectual growth. At the end of the competition, the top three participants will be awarded honorable mentions, acknowledging their exceptional contributions to the field of physiology.
2. Eligibility
2.1. The competition is open to all undergraduate students from any recognized educational institution. Six (6) students from those subscribed will be selected to the final oral presentation. Selected students for the final oral presentation will be notified in advance via email. Confirmation of presence is required to secure their participation.
2.2. Each participant must be the primary author and presenter of the work submitted in the English language.
3. Registration
3.1. Students interested in participating must register online by the specified meeting deadline, providing their personal and academic information, along with an abstract of their research work in English.
3.2. The abstract should include a concise summary of the research question, methodology, results, conclusions, and financial support.
4. Presentation Format
4.1. The competition will be conducted through **5 min oral presentation (5 slides) (5 min talk + 5 min questions)** as the following model:
Slide 1: Title, Authors, Institution
Title of the study: Clearly state the title of the research study.
Names of authors and co-authors: List the names of all the authors and co-authors involved in the study.
Institution: Mention the name of the educational institution or research organization affiliated with the study.

Location: Include the state and country where the institution is located.

Slide 2: Introduction

Briefly introduce the research topic and its significance.
Clearly state the research objectives.
Provide context and background information to engage the audience.

Slide 3: Methods

Highlight the methodology employed in the study.
Provide a concise overview of the research methodology.
Explain the approach, data collection methods, and experimental design.
Mention any specific procedures or techniques used.
Optionally, include visuals or diagrams to aid understanding.
Inform Ethics Committee approval (mandatory)

Slide 4: Results

Present key findings and data in a concise and visually appealing manner.
Use graphs, charts, or tables to effectively convey results.
Highlight significant trends or patterns observed.
Provide an analysis of the results, explaining their significance.
Optionally, include any statistical analysis or measures used.

Slide 5: Discussion, Conclusion, and Financial Support

Discuss the implications of the results and their relevance.
Summarize the main conclusions drawn from the research.
Acknowledge any financial support received for the study.
Mention grants, scholarships, or funding sources that contributed to the research.
Highlight the importance of the financial support in enabling the study and its impact on the research outcomes.

It is recommended that the presentation slides be designed in accordance with the branding guidelines of PANAM 2023 Congress. This includes incorporating the official logo, color scheme, and overall visual identity of the congress to ensure a cohesive and professional presentation. Adhering to the congress branding will not only enhance the visual appeal of the slides but also create a sense of unity and alignment with the event.

5. Presentation Time

5.1. Each participant will have a maximum of 5 min to present their work in a slot of 10 min (5 min talk + 5 min questions) to the evaluating committee.
5.2. Participants must strictly adhere to the time limit and manage their presentation time effectively.
5.3. All presentations and discussions during the competition must be conducted in English.

6. Evaluation

6.1. The evaluating committee will assess the quality and relevance of the research work presented based on scientific content, methodology, results, conclusions and the ability to effectively convey the research within the given time (max 2 min).
6.2. The evaluating committee's decision will be final and cannot be appealed.

7. Awards

7.1. The top three (3) participants with the highest scores will receive an honorable mention for their outstanding achievements. All participants will receive a certificate of participation as proof of their involvement in the competition.
7.2. The awards will be presented during the congress closing ceremony.

8. Code of Conduct

8.1. Participants must maintain a professional and respectful demeanor throughout the competition.
8.2. Any form of plagiarism or academic misconduct will lead to immediate disqualification from the competition.

9. Intellectual Property

9.1. Participants retain full ownership of their research work and intellectual property rights.
9.2. By participating in the competition, participants grant the organizing committee the right to display their presentation for promotional purposes with appropriate attribution.

10. Disclaimer

The organizing committee reserves the right to modify the competition regulations, schedule, or any other aspect deemed necessary. Participants will be duly notified of any changes in a timely manner. By registering for the Scientific Competition Session in Physiological Sciences, participants agree to comply with the regulations and guidelines.

TECHNICAL WORKSHOPS

TW1 - THE JOURNAL OF PHYSIOLOGY WORKSHOP

Coordinator: Kim E. Barret (EiC The Journal of Physiology, USA)
Speakers: Kim E. Barrett (Editor in Chief, *The Journal of Physiology*) • *Distinguished Professor of Physiology and Membrane Biology, UC Davis School of Medicine, USA.*
Ken O'Halloran (Senior Ethics Editor, *The Journal of Physiology*) • *Professor of Physiology, University College Cork, Ireland NI/NP.*
Luis Sobrevia (Regional Editor for Central and South America, *The Journal of Physiology*) • *Professor of Molecular Physiology, School of Medicine, Pontificia Universidad Católica de Chile, Chile.*
Topics: Getting your physiological research published
Opportunities for ECRs in *The Journal*
Allocated time: 30 min
Description
In a workshop aimed predominantly at early career researchers (ECRs), the Editor in Chief, a Senior Editor, and the newly appointed Regional Editor for South and Central America from *The Journal of Physiology* will provide tips on getting your physiological research published, including strategies for writing up your manuscript and how best to appeal to editors and reviewers. The speakers will specifically address the advantages to be gained from submitting your work to *The Journal.* Opportunities for ECRs to get involved with *The Journal* such as our Editorial Board Fellows Scheme and Journal Club articles will also be covered, and there will be plenty of time for questions and answers.

TW2 - BENTHAM SCIENCE WORKSHOP

Coordinator: Frans Letterström (Director of Global Sales, Bentham Science, United Arab Emirates)
Speakers: Frans Letterström (Bentham Science)
Topics: Publishing in Bentham Science's journals
Consortium of national research libraries for LAC
Allocated time: 30 min
Description

TW3 - ELSEVIER WORKSHOP

Coordinator: Rafael Teixeira (Content Acquisition Lead – Biochemistry Journals, ELSEVIER)
Speakers: Rafael Teixeira (ELSEVIER)
Topics: Publisinhg in Elsevier's journals
Allocated time: 30 min
Description

PRECONGRESS COURSES

PCC1 - SHAPING THE FUTURE OF SKELETAL MUSCLE: METHODOLOGIES AND EMERGING FINDINGS

Coordinators: Denisse Valladares (Universidad de O'Higgins, Chile)
Luis Peñailillo (Universidad Andrés Bello, Chile)
Date: Monday 27 November
Allocated time: 3 h (180 min)
Description
This course focused on the latest research methodologies and emerging findings related to skeletal muscle function. The course is designed to give participants an in-depth understanding of the factors affecting skeletal muscle function and how to enhance it. The course is divided into four main sections. The first section covers the epigenetic regulation of skeletal muscle, focusing on DNA methylation and extracellular vesicle-derived miRNAs. The second section covers methods for assessing mitochondrial function in skeletal muscle, including the Oxygraph-2k respirometer and the Seahorse Extracellular Flux Analyzer. The third section focuses on chronic inflammation in skeletal muscle function, including the role of the inflammasome in obesity and insulin resistance, inflammaging and its implications in obesity and sarcopenia, and the fibro-adipogenic progenitors in obese-skeletal muscle. The fourth section covers new muscle function enhancers, such as omega-3 lipid mediators in the muscle regeneration process, eccentric exercise in skeletal muscle, and the possibility of increasing skeletal muscle mass in individuals over 85 years old.
Overall, this course is for students interested in the latest research on skeletal muscle and its function. It aims to provide participants with practical knowledge and skills to enhance muscle function and promote healthy ageing.
Activities and speakers
Section 1: Epigenetic regulation of skeletal muscle function
Bernardo Krause • *Universidad de O´Higgins, Chile.*
Challenges in the study of DNA methylation.
Denisse Valladares • *Universidad de O´Higgins, Chile.*
Impact of skeletal muscle-derived extracellular vesicles and miRNAs.
Section 2: Methods for assessing skeletal muscle function
Matías Monsalves • *Universidad de O´Higgins, Chile.*
Oxygraph-2k respirometer (Oroboros).
Juan Camilo Calderón • *Universidad de Antioquia, Colombia.*
Studying mature skeletal muscle fibers: beyond the Flexor Digitorum Brevis.
Section 3: Chronic inflammation in skeletal muscle function
Paola Llanos • *Universidad de Chile, Chile.*
Role of NLRP3 inflammasome in obesity related low-grade inflammation and insulin resistance in skeletal muscle
Gonzalo Jorquera • *INTA, Universidad de Chile, Chile.*
Inflammaging: Implications in obesity and age-related sarcopenia
Section 4: New muscle function enhancers
Sebastián Jannas • *Universidad de O´Higgins, Chile.*
Unleashing the benefits of omega-3 lipid mediators in the muscle regeneration process.
Luis Peñailillo • *Universidad Andrés Bello, Chile.*
Eccentric exercise in skeletal muscle: good or bad?
Gabriel N. Marzuca-Nassr • *Universidad de la Frontera, Chile.*
Is it possible to increase skeletal muscle mass over 85 years old?.

PCC2 - PRE-CONGRESS TEACHING ONE DAY WORKSHOP (Sponsored by ADInstruments)

Coordinators: Robert G. Carroll (Brody School of Medicine, East Carolina University, USA)
Patricia A. Halpin (University of New Hampshire, Department of Life Sciences, USA)
Fernanda Klein Marcondes (Dept of Biosciences, Piracicaba Dental School, University of Campinas (UNICAMP), Brazil)
Dee U. Silverthorn (Dell Medical School, University of Texas at Austin, USA)
Date: Monday 27 November
Allocated time: 7 h (420 min)

Activities and speakers:

Tatiana Mendes, Patricia Mendes *(ADInstruments)* • ADInstruments - Using active learning methodology for laboratories classes in different models: hybrid, online, or in lab

Paulo Fernando Guedes Pereira Montenegro *(Brazil)* • Basic electronics for physiologists: a tool to create physical manipulatives for teaching purposes

Loreto Véliz *(Chile)*, Carolina Serrano *(Chile),* Victoria Velarde *(Chile)* • Learning physiology and contributing to the community

Robert G. Carroll *(USA)*, Dee U. Silverthorn *(USA*) • Publishing your educational scholarship

Camilo Lellis-Santos *(Brazil)* • Smartphone-assisted experimentation for physiology education

Patricia A. Halpin *(USA),* Helena Carvalho *(USA)* • Using dramatizations in face-to-face and online courses to teach physiology

Fernanda Klein Marcondes *(Brazil),* Luís Henrique Montrezor *(Brazil)* • Using educational games to teach physiology

Chaya Gopalan *(USA)* • Using flipped teaching in underserved colleges to promote student engagement

Tentative schedule (All workshops are 80 min long)

MONDAY 27 NOVEMBER			
Time	**ROOM 2A**	**Time**	**ROOM 2B**
9:15-10:35	Using educational games to teach physiology	9:15-10:35	Using active learning for laboratory classes in different models: hybrid, online, or in lab
10:35-0:45	*BREAK*	*10:35-10:45*	*BREAK*
10:45-2:05	Basic electronics for physiologists: a tool to create physical manipulatives for teaching purposes	10:45-2:05	Using dramatizations in face-to-face and online courses to teach physiology
12:05-1:15	*LUNCH*	*12:05-1:15*	*LUNCH*
1:15-2:35	Smartphone-assisted experimentation for physiology education	1:15-2:35	Using flipped teaching in underserved colleges to promote student engagement
2:35-2:45	*BREAK*	*2:35-2:45*	*BREAK*
2:45-4:05	Learning physiology and contributing to the community	2:45-4:05	Publishing your educational scholarship

Specific details for each activity

Activity:

ADInstruments - Using active learning for laboratory classes in different models: hybrid, online, or in lab

Facilitators: Tatiana Mendes (ADInstruments)

Patricia Mendes (ADInstruments). Email: P.mendes@adinstruments.com

Abstract

ADInstruments' Lt is an award-winning online learning platform with ready-to-use content for life sciences, nursing, and medicine. In the workshop, participants will carry out laboratory experiments using active learning and evaluate the results with the statistical analysis tools of the teaching platform. Some of the editable content created in Lt Kuracloud will be presented as a solution for teaching physiology.

Resources: Internet connection, power plugs for equipment, TV or projector. Desks for at least 3 different stations.

Activity:

Basic electronics for physiologists: a tool to create physical manipulatives for teaching purposes

Facilitator: Paulo Fernando Guedes Pereira Montenegro (Laboratório de Ecofisiologia Animal, Universidade Federal da Paraíba, Brazil). Email: pmonte@dse.ufpb.br

Aims

To recognize the most common electronic concepts and components

To build simple circuits as part of physical models to aid learning in physiology

To engage in a continuous learning process in electronics

Abstract

Physical manipulatives are concrete objects used as models for a given phenomenon, structure or concept, and they foster hands-on experiential opportunities in the classroom. Electronic circuits are one of the most amusing models because they can easily simulate simple physiological stimulus-response pathways. In this workshop, students will be introduced to the basic concepts of electronics, the most commonly used components, wiring diagrams and online resources on electronic materials, projects and circuit simulation. They will then gather in small groups to build simple circuits according to pre-defined diagrams and come up with ideas to use them in manipulative models. At the end of the workshop, it is expected that the participants will be able to recognize the most common electronic concepts and components, and also build simple circuits as part of physical models to aid learning in physiology. We also expect that participants will be encouraged to engage in a continuous learning process in electronics.

Proposed Structure and timing

Part 1 (15 min) – Presentation on electronics theory and common components (slideshow and hands-on activity)

Part 2 (35 min) – Building simple circuits with given components and brainstorm on how to use electronics in physiology teaching

Participation requirements

Participants will be given reading material before the workshop.

Number of participants

Maximum of 30

Activity

Learning physiology and contributing to the community

Facilitators: Loreto Véliz (Faculty of Biological Sciences, Pontificia Universidad Católica de Chile).
Email: lveliz@bio.puc.cl
Carolina Serrano (Faculty of Biological Sciences, Pontificia Universidad Católica de Chile).
Email: cserrano@bio.puc.cl
Victoria Velarde (Faculty of Sciences, Universidad de Valparaíso, Chile).
Email: maria.velarde@uv.cl

Aims

To work on some strategies that will allow you to visualize how you can carry out an activity of A+S in your physiology course

To exemplify the incorporation of reflection and feedback to the social activity developed in the context of the course

To exemplify the development of key transversal skills in your students, such as teamwork, oral communication, and social commitment.

Abstract

Service learning (*aprendizaje+servicio*, A+S) is a teaching-learning method that allows linking the learning objectives of a course into a project that contributes to the community, solving genuine needs in real contexts. In this workshop you will be able to work on some strategies that will allow you to visualize how you can carry out an activity of A+S in your physiology course, incorporating reflection, feedback and promoting the development of key transversal skills in your students, such as teamwork, oral communication, and social commitment.

Proposed Structure and timing

Participants will be divided into groups of 4 people.

Part 1 (10 min) – Introduction. Participants receive the context of the methodology and the objectives that are considered.

Part 2 (10 min) – Reflection activity. In the working groups the participants reflect on situations in different learning contexts.
Part 3 (15 min) – The participants plan, using the selected learning context, an A+S activity that could be carried out, considering one of the learning objectives and/or skills to be developed in a physiology course.
Part 4 (25 min) – Plenary. Each group presents the work that has been done.
Participation requirements
No preparation is needed in advance for the participants.
Room requirements
Markers, small coloured *Post-it* notes and large *Post-it* notes

Activity
Publishing your educational scholarship
Facilitators: Robert G. Carroll (Brody School of Medicine, East Carolina University, USA).
Email: carrollr@ecu.edu
Dee U. Silverthorn (Dell Medical School, University of Texas at Austin, USA).
Email: silverthorn@utexas.edu
Aims
To familiarise attendees with the various formats that manuscript submissions can take
To support colleagues in creating their next submission
To enhance chances of manuscript acceptance
Abstract
Publication of peer-reviewed articles is a meritorious way of increasing scholarly output, gaining international exposure, and is frequently required for career progression of teaching-focused staff. Physiology teachers can publish their innovative teaching methods and teaching-related research in The American Physiological Society journal *Advances in Physiology Education*. This journal offers the optimal platform for publishing scholarly work on teaching and learning of physiology, neuroscience, anatomy and physiology, and pathophysiology, at all educational levels. *Advances* attracts submissions worldwide and has a broad international reading audience because articles are freely available to readers from the time of publication. The workshop facilitators are *Advances* authors, reviewers, and members of the editorial board who will familiarise the participants with the types of articles and the submission and review process. Attendees in small groups will discuss potential educational projects and manuscripts and will have an opportunity to receive feedback on their ideas.
Proposed Structure
Description of the journal (including the Sourcebook of laboratory activities) (10 min)
Types of articles/requirements/do's and don'ts (20 min)
Discussion groups (participant led) on projects and manuscript feedback (40 min)
Summary remarks (facilitator led) (20 min)
Participation requirements
Participants are asked to bring their ideas for educational research projects or manuscripts in preparation for the discussion groups.

Activity
Smartphone-assisted experimentation for physiology education
Facilitator: Camilo Lellis-Santos (Universidade Federal de São Paulo, Department of Biological Sciences, São Paulo, Brazil). Email: lellis.unifesp@gmail.com
Abstract
Smartphones are not just a communication technology but an extension of the bodies and minds of the digital-native generation of students. However, many teachers do not explore the total capacity of smartphones as a didactic tool. In this workshop, attendees will discuss the pedagogical uses of smartphones to improve and facilitate learning. Ideas and lab protocols will be presented to pave discussions on which smartphones can monitor physiological systems. The principles of inquiry-based learning will be introduced in order to inspire attendees to transform the practical content of a course and engage students as scientists through scientific methodology and creativity.
Resources
Internet access and participants' smartphones 7

Activity

Using dramatizations in face-to-face and online courses to teach physiology

Facilitators: Patricia A. Halpin (University of New Hampshire, Department of Life Sciences, Manchester NH USA). Email: Patricia.Halpin@unh.edu
Helena Carvalho (Virginia Tech Carillion School of Medicine, Roanoke VA USA).
Email: helena@vt.edu

Aims

To demonstrate how dramatizations can engage students in learning
To provide the opportunity to create a dramatization to use in your classroom
To illustrate how dramatization can be used in online courses

Abstract

Adding in-class dramatizations to class time is a fun activity in which students act out different roles in a 'play' that simulates a physiological process; it has been demonstrated to effectively teach Starling forces, the cardiac cycle, membrane transport, and cell signaling. Dramatizations are inclusive activities for diverse learning styles as each student in the class has a role to play. Students benefit by increasing their confidence level through active participation in an accessible venue that invites them to ask questions and promotes long-term retention of material. The instructor benefits by being able to identify misconceptions and remediating them immediately. Dramatizations can be used in any level of instruction, are free or with minimal costs, and are adaptable to any class size. This workshop will provide participants the opportunity to create a dramatization they can use in their own courses. At the end of the session, the participants will showcase their newly created dramatization and receive feedback from the other attendees. Examples of dramatizations using Zoom, which can be used in a lecture or an asynchronous online course will be shared.

Proposed Structure

Introduction and group participation in the cardiac cycle dramatization (10 min)
With input from presenters each group will design and perform a group dramatization activity based on participants' needs (30 min)
Demonstration of groups' newly created dramatizations (20 min)
Demonstration of using dramatizations in online classes (5 min)
Debrief, provide feedback and share ideas with all workshop participants (15 min)

Resources

Colored markers, colored paper, scissors and tape that will stick to clothing
Participant requirements
Bring creativity

Room Requirements

Some open space to move or ability to move tables to the side of the room

Activity

Using educational games to teach physiology

Facilitators: Fernanda Klein Marcondes (Department of Biosciences, Piracicaba Dental School, University of Campinas (UNICAMP), Brazil). Email: ferklein@unicamp.br
Luís Henrique Montrezor (Department of Biological Science and Health - University of Araraquara - UNIARA, Brazil). Email: lhmontrezor@uniara.edu.br

Abstract

The aim of this workshop is to present examples of educational games (printed and digital) developed to teach physiology, combined with instructions to promote student engagement, and also with formative assessments. In groups (4 to 6), the participants will receive one education game to solve, and they will analyse and discuss the sequence of activities that are used to provide pre-preparation of students and to evaluate their learning before, during, and after the use of educational games. This workshop includes the educational games: 1) Puzzle of cardiac cycle, and 2) Integrating physiology of synapses, muscle contraction, and autonomic nervous system.

Resources

Participants' laptops (at least one per group)

Activity

Using flipped teaching in underserved colleges to promote student engagement

Facilitator: Chaya Gopalan (Southern Illinois University Edwardsville, Edwardsville IL USA).
Email: cgopala@siue.edu

Aims

To offer faculty development on flipped teaching

To teach physiology that is practical, flexible, effective, and student-centered

To engage participants in learning using examples of various assignments and assessments requiring minimal or no technology to integrate into their classes

Abstract

Rural colleges may need more professional development opportunities and resources for developing innovative student-centered teaching methods to promote critical thinking and student engagement in the classroom. Most students in these rural colleges are under-represented, under-resourced, and come from underserved high schools. Development of innovative teaching methods that are practical, flexible, effective, and student-centered are needed to teach pre-health students physiology. The flipped classroom is a contemporary instructional design with a central focus on student learning both in the classroom through discussion, peer interaction, and engaging activities and outside, using instructor-guided assignments. The proposed workshop will offer faculty development on flipped teaching. Participants will be engaged in learning examples of various assignments and assessments using minimal or no technology to integrate into their classes. The participants are expected to select one of their own courses to incorporate flipped teaching during this workshop.

Proposed Structure and timing

Part 1 (5 min) – A polling activity to learn the teaching methods used by the participants

Part 2 (15 min) – Introduction of the flipped teaching model

Part 3 (15 min) – Group activity to allow course design using new knowledge

Part 4 (15 min) – Discussion and tips for successful implementation of flipped teaching with minimum or no technology

Participation requirements

The participants are expected to select one of their own courses to incorporate flipped teaching during this workshop.

ACTIVITY WITH AND FOR THE COMMUNITY (AWFC)

AWFC1 - CHALLENGES IN CAREER DEVELOPMENT PATHWAYS: WOMEN VERSUS MAN

Coordinator: Alexis A González (Pontificia Universidad Católica de Valparaíso, Chile)
Participants: Alexis A González • *Pontificia Universidad Católica de Valparaíso, Chile.*
Minolfa Prieto • *Department of Physiology, Tulane University, USA.*
Lucienne da Silva Morcillo • *Associate Professor at Universidade Federal do Rio de Janeiro, Brazil.*
Marcela Herrera • *Labidech Análisis Clínicos, Vita Medicina Reproductiva, Chubut, Argentina.*
Pilar Cárdenas • *Pontificia Universidad Católica de Valparaíso, Chile.*

General description

This activity will target the community of Puerto Varas and surrounding areas along with the assistant to the meeting. The activity is based on experience about women and men in sciences and their opportunities in the field of physiology and related areas with focus in new career researchers and PhD student students interested in postdoctoral positions and internships abroad.

AWFC2 - OPEN SEMINAR TO PUERTO VARAS

Coordinator: Luis Sobrevia (Pontificia Universidad Católica de Chile, Chile)
Participants: PANAM meeting attendees and local Community
Speaker: To select from the PANAM's attendees.
Seminar title: To be announced
Place: Puerto Varas

General description

It has to be general, informative, ludic, short, "amazing"
It must be in Spanish (considering that attendees will be mainly local community)

AWFC3 - BRING A BOOK TO PUERTO VARAS'S SCHOOLS

Coordinator: Luis Sobrevia (Pontificia Universidad Católica de Chile, Chile)
Participants: PANAM meeting attendees

General description

All participants of the PANAM Physiological Sciences 2023 meeting are invited to bring a book to the meeting.
The books can be on any topic ("physiology", literature, science, ecology and ecosystems, history, maths, astronomy, science fiction, medicine, poetry, social sciences, philosophy, etc.).
A series of boxes will be signed with the topics to deposit the books at the validation/registration desk during the whole meeting.
A designated group of people from the meeting will help classify and check the books for topics, general conditions, or any other characteristics that could be inappropriate.
During the last day of the meeting, the books will be donated to different primary Schools in Puerto Varas as a legacy and sign of gratitude to the Community from PANAM Physiological Sciences 2023.
This activity is expected to have a permanent impact from Pan-American physiologists on the local Community.

PANAM 2023

Physiological Sciences

Hosted by Chilean Society of Physiological Sciences &
Latin American Association of Physiological Sciences

November 27-30, 2023
Puerto Varas, Chile

Lectures Abstracts

INAUGURAL LECTURE

The Fluid–Mosaic Membrane Model and Membrane Lipid Replacement

Garth Nicolson[1]

[1] *Institute for Molecular Medicine, USA*

The Fluid–Mosaic Model (FMM) has been the accepted general model for cellular membrane structure for the last 50 years. It is the only model that accounts for membrane asymmetry, variable lateral movements, cis- and transmembrane linkages and dynamic associations of membrane components into multimolecular complexes at the nanometer-scale. Because the FMM was based on 1960s data, it cannot explain all of the discoveries in subsequent years. However, its fundamental organizational and dynamic aspects remain relevant to this day. Additional information has now been included, such as cytoskeletal, extracellular matrix and other structures, specialized lipid–lipid and lipid–protein domains, and other configurations that affect membrane dynamics. The presence of specialized membrane domains has significantly reduced the extent of the fluid lipid membrane matrix as first proposed, and membranes are now considered to be less fluid and more mosaic with some fluid areas, rather than a fluid matrix with predominantly mobile components. However, the fluid matrix regions remain very important, especially in the binding and release of lipid vesicles and the uptake of various nutrients. Membrane phospholipids can associate spontaneously to form lipid structures and vesicles that can fuse with cellular membranes and can transport lipids and other nutrients into cells and organelles and expel damaged lipids and toxic hydrophobic molecules from cells and tissues (Membrane Lipid Replacement). This process and the clinical use of membrane phospholipid supplements has important implications for chronic illnesses and the support of healthy mitochondria, plasma membranes and other cellular membrane structures.

Financing: The Institute for Molecular Medicine, Nutritional Therapeutics, Inc., Naturally Plus, Taiwan

Acknowledgments: Thanks to Prof. Gonzalo Ferreira, Dr. Paul Breeding and Robert Settineri

CLOSING LECTURE

Cardiac CaMKII: Past, Present, and Future - Tracing the Journey from Our Laboratory's Experience

Alicia Mattiazzi[1]

[1] *Centro de Investigaciones Cardiovasculares "Dr. Horacio Cingolani", Physiology, Facultad de Ciencias Médicas, Universidad Nacional de La Plata-CONICET, La Plata, Argentina.*

The Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaMKII) is an intracellular protein prevalent at the cell membrane, within the cytoplasm, and in the nucleus of various cells. Encoded by four distinct genes - CaMKIIα/β/γ/ð - each gene exhibits a different expression pattern. CaMKIIα and CaMKII β are predominantly expressed in the brain, governing synaptic functions crucial for learning, memory, and cognition. Conversely, CaMKII γ and CaMKIIð are found in both healthy and diseased hearts, with CaMKIId being the most highly expressed. Activation of the enzyme occurs when Ca^{2+}/calmodulin (Ca/CaM) binds to the autoregulatory subunit, inducing conformational changes that expose the ATP- and substrate-binding sites. Once activated, CaMKII phosphorylates T287 in the autoregulatory domain of the adjacent subunit, allowing for CaM-independent activity following Ca/CaM dissociation (i.e., autophosphorylation). Besides this canonical activation, Ca/CaM-independent activation can be induced by oxidation of Met-281/282, glycosylation of Ser-279, and S-nitrosylation of Cys290. While modest CaMKII activation contributes to physiological and adaptive processes, its overactivation leads to detrimental effects on excitation-contraction and excitation-transcription coupling, resulting in heart failure, exacerbating cardiac ischemia/reperfusion injury, and promoting life-threatening arrhythmias. Consequently, CaMKII has become an attractive therapeutic target. In this presentation, I will share results from our laboratory's research, which transitioned from initially examining the beneficial role of CaMKII, such as its involvement in the fight-or-flight response, to uncovering its detrimental impact on heart function, where it promotes arrhythmias, necrosis, and apoptosis. Finally, I will explore new

facets of our research, highlighting CaMKII as a potential target for therapeutic interventions.

Financing: PIP # 0350 and PIP # 0627 from National Research Council, Argentina; Grant # 11. M210. Ministry of Education, Argentina

Acknowledgments: I would like to express my gratitude to Dr. Luis Sobrevia and the Congress organizers for the opportunity to present my findings at this Pan-American congress. I also thank the colleagues, students, and technicians in my lab who share the CaMKII journey with me.

L1. Embassy of Honduras in Chile Lecture.

Advances in the Application of Artificial Intelligence in Medicine

Mario R. Lanza Santamaría[1]

[1] *National Academy of Sciences of Honduras, Tegucigalpa, Honduras*

Summary: At the intersection of medicine and artificial intelligence, revolutionary advances are occurring that are transforming healthcare and physiology research. Artificial intelligence (AI) offers innovative solutions for accurate diagnoses, personalized treatment and efficient management of health data.

1. Accurate Diagnosis: AI makes it possible to analyze large sets of clinical and radiological data to identify subtle patterns that doctors may miss. This leads to faster and more accurate diagnoses, which is crucial in areas such as early detection of cancer and heart disease.
2. Personalized Treatment: AI algorithms can evaluate a patient's genetic profile and her medical history to develop personalized treatment plans. This not only increases the effectiveness of the treatments but also reduces side effects.
3. Health Data Management: Managing the large amount of health data is a major challenge. AI simplifies the storage, analysis and sharing of medical data, improving care coordination and clinical research.
4. Telemedicine: AI has also enabled the rise of telemedicine, allowing patients to receive medical care online, which is essential in pandemic situations and to reach patients in remote areas.

Conclusions: Artificial intelligence is redefining medicine and physiology by improving diagnostic accuracy, personalizing treatments, facilitating data management, and providing access to high-quality healthcare around the world. Collaboration between doctors and AI experts is essential to realize its full potential and improve the health and well-being of patients.

L2. Operating principles of the brain oxytocin system

Valery Grinevich[1]

[1] *Central Institute of Mental Health, University of Heidelberg, Neuropeptide Research in Psychiatry, Medical Faculty Mannheim, J5, Mannheim, Germany*

Neuropeptides represent a new class of non-canonical neurotransmitters, which dramatically challenge a plethora behavioral and homeostatic functions. Among a hundred of identified neuropeptides, oxytocin remains the best studied molecule due to a great attention of the general public, basic neuroscience researchers, psychologists and psychiatrists based on its profound pro-social and anxiolytic effects. During the last decade, a substantial progress has been achieved in understanding the complex neurobiology of the brain oxytocin system. However, the picture of oxytocin actions remains far from being complete, and the central question remains: “How does a single neuropeptide exert such pleiotropic actions?”. In this lecture, I will tackle this question, demonstrating the anatomical divergence of oxytocin neurons, their numerous central projections as well as their connectivity with the brain ventricular system. In conjunction, I will describe unique composition of distinct oxytocin-sensitive neurons in different brain regions, modulating distinct forms of behaviors. At the end, I will emphasize advantages and great potencies of oxytocin – in comparison to other neuropeptides – for its use for treatment of human mental disorders.

Financing: German Research Foundation DFG (GR 3619/15-1, GR 3619/16-1, SFB Consortium 1158-3, Germany-Israel Excellence Program grant GR 3619/19-1) and the European Research Council ERC (Synergy ERC grant OxytocINspace #101071777).

L3. URSULAB Chile Lecture.

Contextual Tissue Image Cytometry Using AI-Empowered Precision Microscopy

Rupert Ecker[1,2]

[1] *TissueGnostics GmbH, Vienna, Austria*
[2] *Queensland University of Technology, Translational Research Institute, School of Biomedical Sciences, Faculty of Health, Woolloongabba, Brisbane, Australia*

Introduction: While flow cytometry has been available for researchers and clinicians for decades to perform functional analyses on single cells and determine cellular phenotypes of large cell populations in blood, technologies to perform a similar analysis in situ – ie. in the tissue, the actual localization of most immune responses-are relatively new.

Methods: Our research teams at TissueGnostics and Queensland University of Technology have joined forces to combine TissueGnostics' existing tissue cytometry technology platform and established knowhow with innovative AI solutions to establish The Virtual Histopathologist. This represents a tissue cytometry platform that allows to quantify immune responses where they happen – in the tissue.

Results: Tissue Cytometry permits to determine the in-situ phenotype of individual cells as well as histological entities, like glands, vessels or tumor foci. Applications include but are not limited to the exploration of immune responses in situ and the tumor microenvironment and/or the spatial organization of cellular subpopulations. Earlier attempts to analyse single cells in tissue have mostly been subject to visual estimation, or – at best – to manual counting for decades. To better understand the function of inflammatory cells in tumor development, type and number of inflammatory cells and their proximity to glandular/tumor structures have to be analyzed in-situ. Using TissueFAXS™ Cytometry the time-consuming and error-prone human evaluation of stained histological sections can be approached with an observer-independent and reproducible technology platform, offering a high degree of automation, paired with user interaction at relevant points of the analytical workflow.

L4. Skeletal muscle atrophy and age-related loss of muscle mass and function.

Sue Bodine[1]
[1] *Oklahoma Medical Research Foundation, Aging and Metabolism Research Program, 825 NE 13th St, Oklahoma City, OK, USA*

Skeletal muscle wasting is a serious consequence of many diseases and conditions for which there is no treatment. A broad array of stressors can initiate muscle loss including decreased neural activity, decreased external loading, nutritional deprivation, inflammation, elevated glucocorticoids, mitochondrial dysfunction, and oxidative stress. Over the past two decades considerable progress has been made in our understanding of the molecular and cellular mechanisms underlying the loss of muscle mass under different conditions. Interestingly, the upregulation of the E3 ligases MuRF1 and MAFbx seems to be a common early event in many divergent atrophy conditions, e.g. inflammation, disuse, starvation, cancer cachexia, and aging. However, upregulation of MuRF1 and MAFbx is only one component of the atrophy process, and multiple interacting pathways involved in protein synthesis and degradation contribute to the loss of muscle fiber cross-sectional area. The loss of skeletal muscle mass with age differs from acute atrophy in that it is a slow progressive process that does not occur uniformly across all muscles or across fiber types within a muscle. Further, age-related muscle loss is a consequence of multiple factors including inactivity, chronic inflammation, altered proteostasis, mitochondrial dysfunction and denervation; thus, multiple signaling pathways and cellular processes are altered at different times over the course of the aging process. The data reveal that muscle atrophy is not a single disease and that different strategies will likely need to be taken to prevent the loss of muscle mass under these disparate conditions.

L5. Titin: big protein with big responsibilities

Henk Granzier[1]
[1] *University of Arizona, Cellular and Molecular Medicine, Tucson, AZ, USA*

In this presentation, I will focus on recent discoveries regarding the structure and functions of titin. Titin is the largest protein known (Molecular weight 3-4 MDa), and comprises a separate myofilament within striated muscles. The titin molecule spans the half sarcomere, extending from the Z-disk to the M-band of the sarcomere. The C-terminus firmly anchors titin to the Z-disk, while its elastic I-band acts as a molecular spring,

providing passive stiffness to striated muscles. Moreover, recent evidence indicates that the A-band segment regulates the length and activation state of thick filaments, while the M-band section of titin is believed to possess signalosome capabilities due to its kinase domain and interactions with other proteins. The human titin gene (TTN) consists of 364 exons, and its alternative splicing yields various cardiac and skeletal muscle titin isoforms, primarily differing in their molecular spring lengths, and consequently having different passive stiffnesses. Multiple types of post-translational modifications also occur on titin, and these can fine-tune titin's stiffness during normal physiological processes. Posttranscriptional and post-translational processes have been found to be deranged in various myopathies. Pathogenic variations in TTN also contribute to a wide array of cardiac, skeletal, and cardioskeletal disorders at various stages of life. Particularly, heterozygous A-band truncating variants (known as TTNtv) are often linked to dilated cardiomyopathy. My intention is to cover these topics and discuss outstanding challenges.

L6. Neuropeptide neurotransmission in stress physiology in brain and periphery

Lee Eiden[1]

[1] *National Institute of Mental Health, National Institutes of Health, Section on Molecular Neuroscience, Laboratory of Cellular and Molecular Regulation, Building 49, Room 5A-38, Bethesda, United States*

Acetylcholine is classically considered the only first messenger controlling catecholamine discharge from adrenal medulla and sympathetic nerves. More recently, the neuropeptide PACAP (pituitary adenylate cyclase-activating polypeptide), expressed in preganglionic cholinergic neurons, has been invoked as a co-transmitter controlling catecholamine secretion, particularly during stress. In vivo, ex vivo, and cell culture experiments over the past decade have solidified a role for PACAP in mediating stress-induced catecholamine secretion from chromaffin cells of the adrenal medulla, via a novel non-action potential-dependent depolarization signaling chain, a secretagogue mechanism possibly shared with other Gs-coupled GPCRs.

The role of PACAP in stress responding extends to the central nervous system. Circuits from brain stem to extended amygdala, and frontal cortex to hypothalamus, separately mediate endocrine and behavioral responses to various stressors. In these circuits, PACAP is co-stored and released not with acetylcholine as in the periphery, but with the excitatory neurotransmitter glutamate, as well as other neuropeptides. The role(s) of multiple first messengers released from individual neuronal populations during the stress response remain, in most cases, to be elucidated.

Immediate-early genes (IEGs) including fos and egr1/zif26 are markers for neuronal engagement in brain-coordinated physiological responses to both systemic and psychogenic stressors, and delineate the signaling pathways activated by distinct first messengers during physiological responses to stress. Deletion of PACAP from CNS neurons, for example, is linked to fos, but not egr1 induction in hypothalamus and amygdala. The neurotransmitter dependence of IEG induction may reveal the combinatorial code for collaboration of multiple first messengers in circuit-wide stress responding.

Financing: NIMH Intramural Research Program support via MH002386

Acknowledgments: The speaker acknowledges SMN members past and present, and collaborators Limei Zhang at the Autonomous National University of Mexico (UNAM), Corey Smith at Case Western Reserve, and Sarah Gray at the University of Northern British Columbia whose work is mentioned in this talk.

L7. Nitric oxide: a major regulator of the beginning and the end of microvascular permeability in inflammation.

Walter Duran[1]

[1] *Department of Pharmacology, Physiology and Neuroscience, Rutgers-New Jersey Medical School, Newark, NJ 07103, U.S.A.*

The most important function of the cardiovascular system is to bring about the exchange of molecules between blood and tissue to support cell life. We have established that the movement of eNOS [endothelial nitric oxide synthase]) from

cell membrane to cytosol and its production of nitric oxide (NO) is fundamental for the onset of hyperpermeability. We have recently begun to explore the role of NO in the termination of inflammation/agonist-induced hyperpermeability. We apply either platelet-activating factor (PAF) or vascular endothelial growth factor (VEGF) as the inflammation hyperpermeability inducing agent. We observed that both inflammatory agonists initiate a delayed cascade of cAMP-dependent pathways that causes inactivation of hyperpermeability. PAF and VEGF increase cAMP, which leads to Epac-1 activity and VASP phosphorylation (VASP: vasodilation-stimulated phosphoprotein). The activation of Epac-1 causes return of eNOS (coupled to Epac-1) to the cell plasma membrane. The decrease in cytosolic NO is a primary factor in the termination of agonist-induced hyperpermeability.

Financing: (Supported by NIH grant R01 HL146539).

L8. Thermoregulation in extreme environments – lessons learned from studies in sub-Sahara and space

Hanns-Christian Gunga[1], Oliver Opatz[1], Martina Anna Maggioni[1]

[1] *Charité-Universitätsmedizin Berlin, Institute of Physiology - Center for space medicine and extreme environments Berlin, Chariteplatz 1, 10117, Berlin, Germany*

The ideal environment for the human body is a mild climate by the sea, but most people today live and work under different conditions. For professional or tourist reasons, for example, they expose themselves to extreme environments, from overwintering in Antarctica to even fly into space. In this frame, the lecture will specifically focus on core body temperature adaptation to different conditions, in laboratory and field studies. It will also deal with new methodological approaches to measure core body temperature and demonstrate for example their application to monitor the circadian rhythm of core body temperature in astronauts on the International Space Station (ISS). Preliminary analysis of data from the circadian rhythm experiments revealed that the *mesor*, *nadir*, and *amplitude* of core body temperature circadian rhythm in astronauts are altered to varying degrees. The reasons for the altered circadian rhythm are unknown. Finally, the effects of climate change and heat stress on population health and performance in sub-Saharan Africa are discussed in detail, using the methodological repertoire from the experiments on the ISS for research in Africa. These current examples from ongoing research will be used to illustrate how narrow the optimal temperature range for humans is and what technological developments may need to be considered to enable residence in these increasingly extreme thermal stressed areas on our planet in the up-coming decades.

Financing: Funding grants: 50WB1330, 50WB1724, 50WB01730, 50WB2030, and DFG_FOR_GU2936

Acknowledgments: This investigation was supported by the ELIPS 3 and 4 programs of ESA, DLR, and DFG

L9. Competency-based education in physiology

Dee Silverthorn[1]

[1] *University of Texas at Austin, Medical Education, Dell Medical School, Austin, Texas, USA*

Competency-based education (CBE) means designing a curriculum that promotes the development of skills and behaviors in addition to acquisition of knowledge. The World Federation for Medical Education (www.wfme.org) is establishing medical education standards based on CBE, and physiology programs globally are beginning to define their desired competencies and map them onto existing courses. This talk will examine the roles of learning outcomes, backward design, and curriculum mapping in the development of a competency-based physiology course.

L10. Amyloid-β and tau: the trigger and bullet in Alzheimer's disease pathogenesis

George Bloom[1], Andrés Norambuena[2]

[1] *University of Virginia, Departments of Biology, Cell Biology, and Neuroscience, Charlottesville, USA*

[2] *University of Virginia, Department of Biology, Charlottesville, USA*

The defining features of Alzheimer's disease (AD) are cognitive impairment, and the presence in brain of two types of poorly soluble, fibrillar

aggregates: extracellular amyloid plaques and intraneuronal neurofibrillary tangles, which are respectively formed by amyloid-β (Aβ) peptides and the neuronal protein, tau. A major focus of our lab has been defining pathogenic connections between Aβ and tau as seminal factors in AD pathogenesis. We found that extracellular Aβ oligomers (xcAβOs) induce ectopic re-entry of neurons into the cell cycle, a prelude to most neuron death in AD, and mitochondrial poisoning. These phenomena occur by mechanisms that depend on soluble forms of intracellular tau, and involve xcAβO-mediated activation of multiple kinases that catalyze site-specific tau phosphorylation, conformational changes in tau and damage to NMDA receptors. Importantly, all of these effects occur within hours of neuronal exposure to xcAβOs, independently of the incorporation of Aβ and tau into plaques and tangles. A derivative effect of neuronal exposure to xcAβOs may be intracellular production and subsequent release of tau oligomers that thus are extracellular (xcTauOs) and spread tau pathology from neuron to neuron by a prion-like mechanism. Some of our most recent work has focused on responses of neurons to xcTauOs. We found that xcTauOs cause striking invaginations of neuronal nuclei coupled to impaired nucleocytoplasmic transport and altered chromatin structure and gene expression, most notably a dramatic upregulation of tau mRNA levels. xcTauOs might therefore drive a positive feedback loop that stimulates production of excess tau mRNA, and by extension, more xcTauOs.

Financing: NIH/NIA grants RF1 AG051085 and R01 AG067048; the Owens Family Foundation; Alzheimer's Association Zenith Fellowship number ZEN-16-363266 and research grant number 4079; the Cure Alzheimer's Fund; the Stanley E. Fulton Foundation; and the Rick Sharp Alzheimer's Foundation.

Acknowledgments: Bloom lab members and others who contributed to this work include Drs. Matthew Seward, Eric Swanson, Evelyn Pardo, Shahzad Khan, Erin Kodis, Lauren Rudenko, Guillermo Eastman, Binita Rajbanshi, Merci Best, Xuehan (Victoria) Sun, Antonia Silva, Charles Glabe, James Mandell and Gonzalo Ferreira, and Horst Wallrabe.

L11. The central respiratory pattern generator and control of ventilation: New pathways and players

Jan-Marino Ramirez[1,2]

[1] *University of Washington School of Medicine, Neurological Surgery and Pediatrics, Professor, 1900 Ninth Avenue, Seattle, USA*

[2] *Seattle Children's Research Institute, Center for Integrative Brain Research, Director, 1900 Ninth Avenue, Seattle, USA*

The mechanisms underlying breathing have been debated for over a century. Evidence has been presented for synaptic and intrinsic bursting mechanisms, for distributed network mechanisms, and the contributions of localized excitatory rhythmogenic microcircuits. Much of the debate has been centered on the notion that a single, cellular or network mechanism can explain rhythmogenesis and breathing. However, this simplistic idea is difficult to reconcile with the fact that breathing needs to be plastic and is integral to many behaviors. From cardiovascular coupling to vocalization, swallowing, coughing, cognition and emotions, the respiratory network assumes different network configurations, involves multiple cellular mechanisms, and switches between single to multi-phased rhythms to cope with the ever-changing metabolic, physiological and behavioral demands of an organism. Modern transgenic, optogenetic, chemogenetic, transcriptomic and precision multicellular recordings provide unprecedented insights into the multiple mechanisms that govern breathing, cardiorespiratory coupling, and the coordination with other behaviors under physiological and pathophysiological conditions. This presentation will take you on an exciting journey that reflects both the complexity but also the apparent simplicity of breathing to ensure that we stay alive, happy and oxygenated. Viajemos a las maravillas del cerebro.

Financing: This work was funded by the National Institute of Health

Acknowledgments: I want to thank all the amazing members of my laboratory

L12. Incretin regulation of the hypothalamic-neurohypophysial system

David Murphy[1], Danijela Tatovic[2], Michael Greenwood[1]

[1] *University of Bristol, Bristol Medical School, Bristol, United Kingdom*

[2] *North Bristol NHS Trust, Diabetes and Endocrinology Department, Bristol, United Kingdom*

Introduction: Excessive release of arginine vasopressin (AVP) is implicated in many diseases including cardiovascular disease, diabetes, obesity, and metabolic syndrome. Once thought to be a consequence of disease, data now supports a causative role. We have previously identified CREB3L1 as a transcription factor that co-ordinates vasopressin synthesis and release in the supraoptic nucleus (SON) of the hypothalamus.

Objective: To identify mechanisms orchestrated by CREB3L1 that co-ordinate vasopressin release.

Methods: We mined *Creb3l1* knockdown SON RNA-seq data to identify downstream target genes. We investigated the expression of these genes and associated pathways in response to physiological and pharmacological stimulation. We used viruses to selectively knockdown gene expression in the SON and assessed physiological and metabolic parameters. We used phosphoproteomics to identify mechanisms that affect hormone release by the pituitary gland. Animal work was carried out under the auspices of UK Home Office Licence PP9294977.

Results: We identified the glucagon like peptide 1 receptor (*Glp1r*) gene as a CREB3L1 target and found increased expression in stimulated AVP neurones. Selective knockdown of SON *Glp1r*s decreased food intake and body weight. Treatment with GLP-1R agonist liraglutide decreased AVP synthesis and release. Quantitative phosphoproteomics of the pituitary neurointermediate lobe revealed that liraglutide initiates hyperphosphorylation of presynapse active zone proteins that control vasopressin exocytosis. Interestingly, liraglutide mediates phosphorylation of the AVP precursor, with functional consequences.

Conclusion: We show that GLP-1R signalling inhibits the vasopressin system. Our data advises that hydration status may influence the pharmacodynamics of GLP-1R agonists so should be considered in current therapeutic strategies.

Financing: This research is supported by a Medical Research Council grant (MR/W028999/1) to DM, DT and MG.

L13. From the laboratory bench to the patient: pre-clinical and clinical development of a drug for obesity and type II diabetes

Carlos Escande[1]

[1] *Institut Pasteur Montevideo, Laboratory of Metabolic Diseases & Aging, Mataojo 2020, Montevideo, Uruguay*

Obesity-related type II diabetes (diabesity) has increased global morbidity and mortality dramatically. Previously, the ancient drug salicylate demonstrated promise for the treatment of type II diabetes, but its clinical use was precluded due to high dose requirements and concomitant side effects. Recently, we showed that the nitroalkene group of unsaturated nitro-fatty acids can be attached to different molecular scaffolds to confer beneficial drug actions. In this study, we designed a nitroalkene derivative of salicylate, 5-(2-nitroethenyl)salicylic acid (SANA), and assessed its effects in murine diet-induced obesity (DIO). The data show that SANA reduces DIO, liver steatosis and insulin resistance at doses up to 40 times lower than salicylate. Furthermore, SANA showed: a) improved metabolic beneficiary effects when compared to Metformin, and b) weight-loss effects comparable to Liraglutide. Analyses of adipose tissue revealed SANA-mediated stimulation of mitochondrial respiration, via a creatine-dependent heat production pathway. Indeed, depletion of creatine/creatine kinases resulted in the loss of SANA action. Together, these data demonstrate SANA as a candidate for the treatment of obesity and type II diabetes. Based on that, we aimed to follow the pre-clinical development necessary to perform a Phase IA/B clinical trial in human subjects. SANA complied with all the required safety requirements from regulatory agencies, and is currently being administered to human subjects. Preliminary Pk analysis in humans suggests that efficacy doses could be achieved without toxic effects.

Financing: ANII, Eolo Pharma
Acknowledgments: ANII, PEDECIBA

L14. Chilean Society of Physiological Sciences Lecture

Hypertension, diabetes, and the kidney

Victoria Velarde[1]

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The kidneys as part of the urinary system have several important functions in our body and can be damaged by several diseases, in particular hypertension and diabetes. These diseases can damage the kidney in its vascular component, the tubular component or in the filter where the vascular and the tubular components come together. To understand the effect of these diseases in the kidney several models can be used including cellular cultures and whole individuals.

In this talk I will show you studies performed by some of my students using different models that reflect the combination of their own interests with mine. In the first study we evaluated the effects of high glucose on the expression of B1KR and its associated signaling in endothelial cells. In the second study, we evaluated the effect of DHEA on the aggregation of platelets from postmenopausal women with type 2 diabetes. On the third study we demonstrated the beneficial effects of boldine on renal damage in diabetic rats. On the fourth study we evaluated young subjects with enlarged waist circumference and dyslipidemia but without type 2 diabetes or hypertension, for markers associated with a higher risk of cardiovascular diseases. On the fifth study we assessed the effect of boldine on the progression of kidney disease in the renal hypertensive rat model 2K1C.

Finally, I discuss the essential information that an average individual should have regarding the kidneys to acknowledge their importance and take proper care of them.

L15. Leptin and Control of Breathing

Vsevolod Polotsky[1], Xin Wang[2], Mateus Amorim[1], David Mendelowitz[2]

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Introduction: Leptin suppresses food intake and increases metabolic rate. In leptin deficient obese *ob/ob* mice, leptin augments hypercapnic sensitivity. We hypothesize that LEPR^b+ neurons in the DMH stimulate breathing and upper airway patency during sleep relieving.

Methods: We measured breathing across sleep/wake states, the hypercapnic ventilatory responses (HCVR), CO_2 production, and O_2 consumption in DIO *Lepr^b-Cre* mice expressing DREADDs in LEPR^b+ neurons of the DMH. Optogenetic studies in brain slices revealed numerous projections from DMH LEPR^b+ neurons to serotonergic (5-hydroxytryptophan, 5-HT+) neurons of the dorsal raphe (DR). The role of the novel DMH LEPR^b+-DR pathway in matching breathing and metabolism was further tested in 'loss of function' experiments in DIO *Lepr^b-Cre* mice, in which retrograde AAV harboring *Cre*-dependent caspase was administered into the DR nucleus.

Results: In DIO male mice, activation of LEPR^b+ neurons in DMH increased the metabolic rate and hypercapnic sensitivity and augmented breathing during sleep. Furthermore, activation of the specific population of LEPR^b+-DMH neurons projecting to the serotonergic dorsal raphe nucleus (DR) is necessary and sufficient for these responses. We have further shown that effects of leptin on upper airway patency and breathing during REM sleep are exclusively mediated by the LEPR^b+ DMH neurons projecting to DR, whereas other pathways likely contribute to respiratory effects of leptin during NREM sleep.

Conclusion: Leptin acts on LEPR^b+ neurons in DMH projecting to serotonergic DR neurons are essential to stimulate breathing and enhance the hypercapnic ventilatory response during sleep and wakefulness and maintain upper airway patency during sleep.

Financing: NIH R01 HL128970, NIH R01 HL133100, NIH R41 HL167326

L16. Nuclear positioning and mechanotransduction in health and disease

Gregg Gundersen[1], Susumu Antoku[1], Megane Rayer[1], Keeley Mui[1], Paige Wilson[1], Johnny Rockenbach[1]

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The nucleus is connected to the cytoskeleton through a two protein LINC (linker of nucleoskeleton and cytoskeleton) complex composed of nesprin and SUN proteins in the outer and inner nuclear membrane respectively. SUN proteins anchor to the nuclear lamina. We showed a role for the LINC complex in the nuclear movement that polarizes migrating fibroblasts in culture (Luxton, G et al, Science, 2010). This was the first cultured cell system to identify a function for the LINC complex. We have used it to identify how LINC complexes assemble higher ordered arrays, how lamin A disease variants affect nuclear movement, and to identify additional proteins involved in nuclear movement, including the nesprin-2 associated formins FHOD1/3. We have extended our studies to breast cancer, where the LINC complex is downregulated and to cardiomyopathy where mutations in lamin A and FHOD3 are associated with the disease. We find that downregulation of the LINC complex disrupts formation and maintenance of breast acini structure in 3D culture. With a nesprin-2 degron system, we observe that disruption of acinar structure results from a rapid imbalance in mechanical forces. In a lamin A mouse model of cardiomyopathy, we find that nuclear positioning and shape are disrupted and a feedback mechanism exists involving FHOD3 phosphorylation to limit further nuclear damage. We find that FHOD3 mutants that cause cardiomyopathy disrupt coupling of the nucleus to the actin cytoskeleton. Overall, our studies point to the nucleus as being an active mechanical-engaged organelle that is at the center of cellular mechanotransduction.

Financing: This work was funded by NIH grants to G Gundersen: R35 GM12858593, R01 HL159389 and U01 CA225566

L17. The role of the International Science Council for the progress of science and knowledge in the Americas.

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*T*he International Science Council (ISC) federates more than 250 science organizations encompassing science academies in the areas of natural as well as social and human sciences, research council, the international disciplinary bodies and the young academies. As the major non-governmental body promoting science from discipline-based science all the way to transdisciplinarity, the ISC accompanies its members in anticipating and steering the evolution of science and science systems. Facing unforeseen rapid developments such as artificial intelligence and events such as pandemics, should science merely react and mitigate? or rather should science exercise foresight, reduce risk and maximize opportunities through co-design of science activities with multiple stakeholders? While ensuring that science remains independent and faithful to integrity in the way it is conducted (the ISC is the custodian of the principle of freedom and responsibility of science), the Council is called increasingly to interface with and provide advice to governments, from the national level to the multilateral system. Finally, there is a continuous need to coordinate the science agenda by bridging networks and epistemologies. Present and active in the Latin America and Caribbean region through its office in Colombia and a committee of 15 representatives of ISC members from the whole region, how can the ISC best capture the views, aspirations, experiences and energy of the active scientific community in Latin America and the Caribbean, including its young scientists and young science organizations? These questions will be tackled through an interactive presentation with the audience.

PANAM 2023

Physiological Sciences

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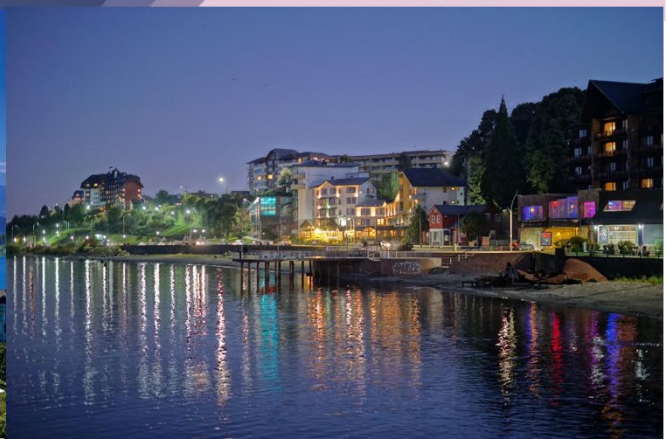
Puerto Varas, Chile

Symposia Abstracts



SS1. SATELLITE SYMPOSIA ROLE OF THE INTERNATIONAL COUNCIL OF SCIENCES AND ACADEMIES OF SCIENCES IN ACADEMIC DEVELOPMENT

In memory of Prof Enrique Forero

(Sponsored by the National Academy of Sciences of Honduras, Embassy of Honduras to Chile, and International Science Council Regional Focal Point for Latin America and the Caribbean, ISC RFP-LAC)

SS1-1

Transforming Higher Education with Artificial Intelligence

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Introduction: In the era of the digital revolution, higher education faces unprecedented challenges and opportunities. Artificial intelligence (AI) is emerging as a powerful ally in the search for more effective and personalized teaching. This presentation explores how AI is transforming higher education and benefiting students, faculty, and administrators.

Benefits of AI in Higher Education:

1. Learning personalization (Adaptive Learning): AI allows the creation and adaptation of content, learning methods and assessments according to the individual needs of students, maximizing their learning and cognitive load.
2. Performance Prediction (Big Data & Learning Analytics): Through data analysis, AI can anticipate academic difficulties and provide early interventions to improve student success.
3. Administrative Efficiency: The automation of administrative tasks allows institutions to focus on higher value activities, such as improving educational quality.

Challenges and Ethical Considerations:

We will address concerns related to data privacy, equity in access to education, and the need to train teachers and administrative staff.

Conclusions:

AI is a catalyst for the transformation of higher education. Collaboration between technologists, educators and academic leaders is essential to realize its full potential and ensure it remains an ethical and effective resource for future learning. Higher education can, and should, lead the way in applying AI for the benefit of society.

S1. EXTRACELLULAR VESICLES FOR DIAGNOSIS AND THERAPY

Chairs: Patricia Rocco (Federal University of Rio de Janeiro, Brazil), Maroun Khoury (Universidad de los Andes, Chile)

S1-1

Extracellular vesicles: New challenges to understanding and treating diseases

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Chagas disease, caused by the protozoan parasite Trypanosoma cruzi, remains a significant public health concern in Latin America and beyond. Despite decades of research, understanding the intricate pathogenesis and developing effective treatments for Chagas disease continue to pose substantial challenges. In recent years, extracellular vesicles (EVs) have emerged as a novel avenue of investigation in the context of Chagas disease. EVs are membrane-enclosed vesicles secreted by various cell types, containing a cargo of proteins, lipids, and nucleic acids, which play essential roles in intercellular communication and the modulation of host-pathogen interactions. These abstract the current state of knowledge on the role of EVs in Chagas disease, shedding light on the complexity of their function in disease pathogenesis. EVs released by both the parasite and host cells have been implicated in immune evasion, tissue damage, and disease progression. Furthermore, these tiny vesicles have shown potential as biomarkers for early diagnosis and disease monitoring, offering a promising tool for improving clinical management. The challenges and opportunities that EVs present in Chagas disease research and treatment. It underscores the need for a comprehensive understanding of the diverse roles of EVs and their cargo in the context of host-parasite interactions. Additionally, it highlights the potential of EV-based biomarkers for early diagnosis and monitoring of Chagas disease, which could significantly enhance patient care. However, significant hurdles, such as standardization and validation, must be overcome

to translate these discoveries into practical clinical applications.

Financing: FAPESP 2020/07870-4

S1-2

Extracellular vesicles are source of prognostic markers in head and neck cancer

Adriana Paes Leme[1]

[1] *Brazilian Center for Research in Energy*

NOTE: This talk was cancelled by the chair (Nov 23, 2023)

S1-3

Therapy with extracellular vesicles in respiratory diseases

Patricia Rocco[1]

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Mesenchymal cells (MSCs) can be obtained from various sources, such as bone marrow, adipose tissue, and umbilical cord. They regulate the immune system, reduce inflammation, and promote tissue repair, which gives them remarkable therapeutic potential and makes them an appealing therapeutic option for various diseases. However, in several clinical trials, MSC therapy has failed to show any benefit. The release of extracellular vesicles (EVs) are mechanisms by which MSCs exert their positive effects. Our group found that therapy with EVs from MSCs reduced inflammation and pulmonary fibrosis in models of acute respiratory distress syndrome (ARDS), asthma and emphysema. However, it is not known whether EVs from differently sourced MSCs would cause a similar beneficial response in respiratory diseases. The decision to use EVs instead of MSCs should ensure greater predictability and reproducibility of results, as EVs can be produced on a large scale and characterized as a pharmaceutical product, unlike MSCs, whose *in vivo* activity is influenced by the individual microenvironment. During this presentation, the treatment of respiratory diseases with EVs will be described. Given the beneficial results of EVs in experimental ARDS, a phase I/II clinical study will be conducted in patients with ARDS (already IRB-approved; ClinicalTrials.gov: NCT06002841).

Financing: CNPq, FAPERJ, CAPES

S2. OBESITY: CHALLENGE OF THE FUTURE

Chairs: M Alicia Carrillo-Sepulveda (New York Institute of Technology, USA), Jennifer Thompson (University of Calgary, Canada)

S2-1

Obesity and its Vascular Complications

M.Alicia Carrillo Sepulveda[1]

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According to the World Health Organization, the prevalence of overweight and obese individuals has increased tremendously over the past four decades affecting nearly 66% of the U.S. population. Despite growing efforts to decelerate obesity rates, the obesity epidemic continues to intensify in the U.S and it is estimated that h-alf of adult Americans will have obesity by 2030. Obesity accounts for more than 70% of cases of essential hypertension, a silent killer and a leading cause of premature death. Unfortunately, there is no cure for hypertension yet, which make the treatment for controlling blood pressure of utmost importance. The mechanisms governing obesity-related hypertension remain unresolved. Currently, there are no antihypertensive medications designed to treat hypertension in obese patients and targeted therapy to treat this at-risk population is urgently needed. Peroxisome proliferator-activated receptor gamma (PPARg), a central metabolic regulator, participates in the pathophysiology of obesity and its vascular complications. PPARg is hyperacetylated in obesity. By using, a unique genetically engineered deacetylation-mimetic mice with a double lysine to arginine mutation (K268R, K293R; called 2KR mice), we found that PPARg deacetylation potentiates endothelial function and delay the development of arterial stiffness in a model of western diet-induced obesity. Thus, our central hypothesis is that PPARg hyperacetylation pathway contributes to vascular complications in obesity. Thus, PPARg deacetylation would be expected to protect against obesity-related

vascular complications. In this symposium, we will present our new findings that support a therapeutic benefit of PPARg deacetylation in obese patients suffering from hypertension.

Financing: NIH - National Heart, Lung and Blood Institute

Acknowledgments: NIH - National Heart, Lung and Blood Institute

S2-2

Obesity and kidney injury: albuminuria and pathogenesis of diabetic kidney disease

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Obesity is a growing public health problem associated with high rate of mortality and morbidity. Moreover, it exhibits correlations with other chronic conditions, such as diabetes, which affect multiple organs, including the kidneys. Diabetes is the primary cause of chronic kidney disease, carrying a substantial financial burden due to its treatment costs and an elevated risk of adverse health outcomes. While glomerulopathy is recognized as a hallmark of diabetic kidney disease (DKD), tubulointerstitial fibrosis is associated with the loss of renal function. Two hypotheses have emerged to explain the origin of DKD, both based on the primary disturbance: glomerulocentric and tubulocentric. The former suggests that initial changes occur in the glomeruli, whereas the latter suggests that tubular damage may precede glomerular damage. Even in the absence of glomerular dysfunction and injury, albuminuria can manifest in the early stages of diabetes. Some authors have demonstrated, in both diabetic animal models and patients, that albuminuria can occur even without alterations in glomerular albumin permeability, pointing to a tubular origin. Furthermore, some studies have underscored the role of the protein machinery involved in albumin reabsorption in proximal tubule epithelial cells (PTECs) in the development of tubulointerstitial injury observed in kidney disease. In this symposium, we will explore findings related to the potential modulation of albumin endocytosis by high glucose influx in PTECs and its role in albuminuria and tubulointerstitial injury observed in the early stages of diabetes.

Financing: CAPES, CNPq and FAPERJ

Acknowledgments: The author thanks Ms. Giulianne Serpa (TCT fellowship/FAPERJ) for her excellent technical support.

S2-3

Skeletal muscle atrophy and mitochondrial dysfunction in a heart failure with preserved ejection fraction-obesity model

Bianca Nieblas[1,2], Jorge Fragoso[2], Emanuel Guajardo-Correa[2], Selma Romina López Vaquera[2], Armando Osorio[1], Hugo Alves Figueiredo[2], Abraham Méndez-Fernández[1,2], Gerardo García-Rivas[1,2], Noemí García[1,2]

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Introduction: Obesity is the main risk factor associated with Heart Failure with preserved ejection fraction (HFpEF). Exercise intolerance, an early and key manifestation of HFpEF, has been attributed to skeletal muscle atrophy related to mitochondrial dysfunction.

Objective: Evaluate mitochondrial function and atrophy of skeletal muscle in a preclinical HFpEF mice model.

Methods: all procedures were approved by the CICUAL (Protocol #2022-012). 8-week-old C57BL/6 (n=40/time point) mice were randomly assigned to a HFpEF (n=20, 60% kcal high fat diet + L-NAME 0.5 g/L) or a control (n=20) group for 5, 8 and 12 weeks. Characterization of the HFpEF model included phenotypic, biochemical, cardiac function and exercise tolerance evaluation. In gastrocnemius (GM) and soleus muscle (SM), mitochondrial quality control, atrophy genes and mitochondrial function was assessed. Unpaired t-

test was used for the comparisons between groups. Mean ± SEM, alpha $p<0.05$.

Results: while systolic ejection fraction had no changes (HFpEF 63.5±3.1; Control 65.1±2.6$p>0.7$); diastolic dysfunction (8.4±0.3; 5.7±0.5$p=0.002$) and cardiac hypertrophy (10.1±0.4; 9.2±0.1$p=0.03$) are present from 8 weeks. Glucose intolerance (42201.6±1744.7; 33313.8±1309.8$p=0.0007$) and changes in plasma BNP (473.5±57.8; 254.1±12.1$p=0.02$) as well as exercise intolerance (61.3±3.2; 70.8±1.7$p=0.01$) are evident from 8 weeks, as well as lower cell area in GM fibers (846.7±198.1; 1553±87.5$p=0.03$). At 12 weeks, OXPHOS-associated oxygen consumption rate (OCR) (SM 7.9±1.8; 17.5±3.9$p=0.02$, GM 6.5±1.5; 17.7±5.6$p=0.04$), upregulation of pink1 in GM (2.2±0.2; 1.0±0.2$p=0.02$) and downregulation of pgc1a in SM (0.5±0.06; 1.0±0.13$p=0.009$) suggest mitochondrial alterations.

Conclusion: HFpEF development is linked to skeletal muscle functional and phenotypic perturbations in both glycolytic and oxidative fibers.

Financing: Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) and the Institute for Obesity Research, Tecnológico de Monterrey.

S2-4

Maternal obesity is associated with metabolic endotoxemia and changes in the phenotype of hematopoietic progenitor cells and monocytes of their offspring at birth.

Macarena Lépez[1,2], Cristina Silva[3], Ignacio Wichmann[4,5], José A. Castro-Rodríguez[6], Torsten Plösch[7], Paola Casanello[4,8]

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[8] *Pontificia Universidad Católica de Chile, Department of Neonatology, Faculty of Medicine, Santiago, Chile*

Introduction: Maternal-obesity is characterized by metabolic-endotoxemia (elevated blood lipopolysaccharides levels) and is associated with immune dysfunction in the offspring. Monocytes are immune cells that are derived from hematopoietic progenitor cells (HPCs) and develop in early embryonic stages. Thus, the study of these cells would help identify the origin of fetal immune programming by maternal-obesity.

Objectives: To evaluate whether maternal-obesity is associated with metabolic-endotoxemia, changes in immunophenotype, gene expression, DNA-methylation patterns, and function of HPCs and monocytes from their offspring at birth, compared to normal-weight women (NW).

Methods: Pregnant women with maternal-obesity and NW (n=21, both groups) were recruited (ethical-approval #200920001). At delivery, umbilical-cord blood (UCB) was collected to determine lipopolysaccharide levels (photometry), and HPCs and monocytes were isolated to measure immunophenotypes (flow-cytometry), transcript levels (RT-qPCR), global DNA-methylation (EPIC-850K,Illumina®), PPARγ-methylation (pyrosequencing), and *invitro* lipopolysaccharide effect (RT-qPCR). Statistics: group comparisons (Mann-Whitney test), EPIC array (Limma-voom, normalized M-values), significant ($p<0.05$), and FDR-adjustment.

Results: Compared to NW, the maternal-obesity group had higher lipopolysaccharide levels in their offspring. Also, have a greater number of UCB-HPCs early myeloid lineage, and UCB-monocytes with elevated transcripts levels of IL-6, IL-1β, and MCP1, decreased PPARγ levels, global DNA hypomethylation, and a blunted response to

lipopolysaccharide *invitro*. Both UCB-monocytes and HPCs, had higher PPARγ gene methylation.

Conclusion: The offspring of women with obesity present metabolic-endotoxemia, and phenotypic, epigenetic, and functional changes in their immune cells, which suggest a fetal programming by maternal-obesity. Our data provide a molecular argument for including preconception interventions to prevent the effects of maternal-obesity on the offspring.

Financing: Fondecyt 1171406 & 1221812 (PC)DOHaD Brain Mobility award 2021 (ML) Pediatric PUC 2021 (PC, ML)SOCHED 2021-11 (PC, ML) Fondecyt 1141195 (JAC)

S3. OVERVIEW OF PLACENTA – BRAIN AXIS IN PREGNANCY COMPLICATIONS

Chair: Carlos Escudero (Universidad del Bío-Bío, Chile)

S3-1

Impaired brain angiogenesis in offspring from preeclampsia. What we have learnt from preclinical models.

Carlos Escudero[1,2]

[1] *Universidad del Bio Bio, Basic Sciences Department, Faculty of Sciences, Andres Bello #720, Chillan, Chile*

[2] *Group of Research and Innovation in Vascular Health, Chillan, Chile*

Introduction: Children from women with preeclampsia have an increased risk of cognitive and behavioral alterations via unknown pathophysiology. We hypothesize that preeclampsia generated reduced brain cortex angiogenesis in the offspring.

Methods: The preeclampsia-like mouse model (PELS) was generated by administering the nitric oxide inhibitor L-NAME. Confirmatory experiments were done using two additional PELS models. In-vitro analysis used mice and human brain endothelial cells exposed to serum of postnatal pups (P5) or umbilical plasma from preeclamptic pregnancies, respectively.

Results: We report a significant reduction in the brain perfusion and the area occupied by blood vessels in the motor and somatosensory brain cortex of offspring (P5) from PELS compared to uncomplicated control offspring. These data were confirmed using two additional PELS models. Furthermore, circulating levels of critical pro-angiogenic factors, vascular endothelial growth factor (VEGF), and placental growth factor (PlGF) were lower in P5-PELS. Also, we found lower VEGF receptor 2 (KDR) levels in mice and human endothelial cells exposed to the serum of P5-PELS or fetal plasma of preeclamptic pregnancies, respectively. These changes were associated with lower *in vitro* angiogenic capacity, diminished cell migration, larger F-actin filaments, lower number of filopodia, and lower protein levels of F-actin polymerization regulators in brain endothelial cells exposed to serum or fetal plasma of offspring from preeclampsia.

Conclusion: Offspring from preeclampsia exhibited diminished brain cortex angiogenesis, associated with lower circulating VEGF/PlGF/KDR protein levels, impaired brain endothelial migration, and dysfunctional assembly of F-actin filaments. These alterations may predispose to structural and functional alterations in long-term brain development.

Financing: Fondecyt 1200250

Acknowledgments: We acknowledge to all co-authors and collaborative work from the two original papers Lara et al., 2022 and Troncoso et al., 2023

S3-2

Placenta and maternal mental health during COVID-19 pandemic. Focus in Latin America

Marcelo González-Ortiz[1,7], Patricio Castro[6], Pablo Vergara-Barra[3,4], Patricia Huerta[2], Carlos Escudero[5,7]

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[7] *Group of Research and Innovation of Vascular Health (GRIVAS), Chillán, Chile*

Introduction: The COVID-19 pandemic has impacted many aspects of health and society worldwide. A vulnerable group during the pandemic was pregnant women, who were considered to have potentiated risk factors. In physiological pregnancy, maternal systems have several changes and adaptations to support fetal development. These changes involve regulations of cardiovascular, respiratory, and immunologic systems, among others, which SARS-CoV-2 could severely alter.

Objective: To analyse the evidence about COVID-19 pandemic effects on placenta function and maternal mental health.

Methods: Review of the literature, combined with experimental analysis of placenta from COVID-19 patients. Healthy controls (n=6) and gestational COVID-19 cases (n=17) were obtained during the pandemic period, before massive vaccination (Ethics committee certification: CEC-SSC 20-11-60). Placental samples were analyzed by histology and immunohistochemistry to evaluate vascular alterations, inflammation, and oxidative stress.

Results: Our experimental findings showed placental inflammation, oxidative and nitrosative stress, and significative alterations of vasculo-syncytial structure in placental villi in COVID-19 cases, especially in pregnant women with severe COVID-19. On the other hand, the review of the published evidence showed significant increases in anxiety and depression of pregnant women, with higher levels in the Latin-American population compared with European.

Discussion: The conditions of Latin American pregnant women during the COVID-19 pandemic were harder than analogous populations in Europe or North America, which was reflected in higher rates of perinatal anxiety and depression. Still, there is no direct correlation between maternal mental health and placental dysfunction, but there is evidence about prenatal anxiety/depression and plasma inflammatory markers that could impact placenta.

Acknowledgments: Acknowledgements: To the patients and personnel of Hospital Clínico Regional Guillermo Grant Benavente (Concepción, Chile) during the COVID-19 pandemic and the support of our families.

S3-3

Potential mediators involved on the cerebrovascular complications of preeclampsia

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The cerebrovascular complications of preeclampsia account for 40 % of the maternal deaths, most of them occurring in low and middle-income countries. Evidence from clinical studies has been mostly consistent in demonstrating that preeclamptic women have a higher risk of developing acute cerebrovascular complications including eclampsia, hemorrhagic and ischemic stroke, edema, brain herniation, posterior reversible encephalopathy syndrome, and reversible cerebral vasoconstriction syndrome.

The pathophysiology is thought to involve the participation of anti-angiogenic and pro-inflammatory components, but the mechanisms have been mostly characterized from animal preclinical models. Our research group has been working on the subject for over eight years, employing an interdisciplinary approach that considers the use of animal models, cell-based models and clinical data. This approach has proved to be useful in terms of expanding (and challenging) the scope of the current hypotheses while reassessing the role of the placenta-brain axis.

The aim of this symposium is to provide an overview of the current state-of-the-art, in order to generate new research questions on the subject that hopefully would encourage further collaborative work.

Financing: This symposium is self-funded.

Acknowledgments: The speaker would like to acknowledge the support from the grants ANID-PCI REDI170373 (Dr. Pablo Torres-Vergara), ANID-FONDECYT 1200250 (Dr. Carlos Escudero) and STINT MG2019-8462 (Dr. Anna-Karin Wikström).

S3-4

It takes two to tango: Widening our understanding of the origin of schizophrenia from a neurovascular perspective.

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Schizophrenia (SZ) is a chronic debilitating neuropsychiatric disorder that originates during embryogenesis. Increasing research has proposed SZ as a systemic disease with vascular involvement and blood-brain barrier (BBB) dysfunction. Neurogenesis and blood-vessel formation occur in an intermingled and coordinated fashion during development. BBB characteristics reinforce through enhanced intercellular communication among cells of the Neurovascular Unit (NVU), some of which differentiate into mature phenotypes concomitantly to BBB maturation. Through stem cell modeling, we have studied the formation of the BBB in SZ by analyzing the contribution of the main cellular components of the NVU, aiming to decipher a possible neurovascular dysregulation at the onset of SZ. SZ hiPSC-derived Brain endothelial cells (BEC) present an intrinsic failure in proper barrier function. SZ hiPSC-derived astrocytes reveal a chronic inflammatory profile with broad effects on their secretome resulting in vascular deficiencies when assayed both *in vitro* and *in vivo.* In addition, we observed altered expression of genes involved in angiogenesis and synaptic function during SZ neurodevelopment, leading to reduced neuronal network dynamics in the SZ hiPSC-derived neuronal cultures. Altogether, our data suggest that SZ not only presents specific neuronal alterations but may result from a combination of intrinsic deficiencies in the cellular function of BECs and astrocytes at the BBB, resulting in an altered systemic co-dependence in the NVU. Current investigations aim to correlate our results with clinical research to understand the molecular and cellular mechanisms underlying deviations in SZ patients for the development of novel therapeutics.

Financing: Fondecyt 1221522

S4. THE INTERSECTION OF METABOLIC AND INFLAMMATORY MECHANISMS UNDERLYING CARDIOVASCULAR DISEASE; EMERGING EVIDENCE OF SEX DIFFERENCES

Chairs: Patricia Molina (Louisiana State University Health Sciences Center, USA), Heddwen Brooks (Tulane University School of Medicine, USA)

S4-1

CNS regulation of metabolism

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The regulation of energy homeostasis including glucose metabolism, involves an exchange of information between the nervous systems and peripheral organs and tissues; therefore, altering central and/or peripheral neural pathways could be an alternative solution to modulate whole-body metabolism. Liver glucose production and storage are major mechanisms controlling glycemia, and the sympathetic nervous system plays an important role in the maintenance of hepatic glucose homeostasis. Pre-sympathetic neurons in the brainstem and hypothalamus govern the sympathetic output to the liver and change in neuronal activity is one of the underlying mechanisms of autonomic imbalance; therefore, modulation of the excitability of neurons involved in autonomic outflow governance has the potential to improve glycemic status. The symposium talk will provide an overview of the brain - liver pathway and neuronal plasticity during diabetic conditions.

Financing: NIDDK122842 and NIA P01AG071746 - 8456

S4-2

Immuno-metabolic mechanisms of alcohol-associated metabolic instability and their contribution to aging comorbidities

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Alcohol misuse is a major contributing factor to global health burden through its impact on multiple diseases, including cardiometabolic disease. The mechanisms underlying alcohol-induced tissue and organ injury are complex and, in some cases, synergistic. Our emerging data indicate that alcohol serves as an accelerator for aging, particularly in vulnerable populations, like persons living with HIV (PWH). The aging process includes key alterations in physiological processes that impacted by environmental and behavioral factors leads to genomic instability, loss of telomeres, epigenetic changes, loss of proteostasis, dysregulation of nutrient sensor pathways, mitochondrial dysfunction, cellular senescence, and stem cell exhaustion. These cellular alterations underly the frailty phenotype; characterized by decreased resilience and increased susceptibility to diseases associated with aging including cancer, neurodegenerative, and cardiometabolic disease. Our data from studies in non-human primates, humans, and isolated cells strongly suggest that altered immune and skeletal muscle bioenergetics are an explanatory mechanism for the enhanced loss of functional skeletal muscle mass and systemic metabolic dyshomeostasis that characterizes the frailty phenotype. Moreover, our data show that lifetime alcohol use is positively associated with frailty in PWH. We propose that alcohol-associated immunometabolic alterations contribute to accentuated or accelerated progression to a frailty phenotype. Data presented shows that alcohol, HIV, and antiretroviral therapy, work as cellular stressors altering mitochondrial signaling and producing mitochondrial adaptive and maladaptive responses that we believe underlie the alterations in skeletal muscle functional mass, increased viral replication, and decreased viral clearance which together can contribute to increased morbidity and mortality in the infected host.

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S4-3

Sex differences in vascular inflammation

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Over the last several decades, it has become apparent that vascular and endothelial inflammation plays a critical role in the development of numerous disease states including cardiovascular disease, hypertension, and autoimmunity. Moreover, there is a critical role of sex as a biological variable in the pathogenesis of cardiovascular disease and hypertension. Our data demonstrate that renal endothelial cells have the immunological machinery to present antigens directly to immune cells and promote vascular activation and inflammation in a model of hypertension. Moreover, we have found that pathophysiological mechanical forces, including endothelial cell stretch, are key regulators in promoting the upregulation of immunological synapse proteins in a sex-dependent manner. We performed RNA sequencing on primary male and female renal endothelial cells to delineate the genes and pathways associated with this sex-dependent regulation of the endothelial immunological synapse in hypertension.

Financing: Veterans Affairs Biomedical Laboratory Research & Development Career Development Award (CDA-2) 1IK2BX005605-01

S5. CROSS TALK BETWEEN DIFFERENT ORGANS

Chairs: Pedro Leme (Federal University of Rio de Janeiro, Brazil), Marcio Moraes (Federal University of Minas Gerais, Brazil)

S5-1

Cross talk between lung and brain

Pedro Leme Silva[1]

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In the past, the study of physiology was mainly guided by the study of specific organs in an independent way. However, nowadays physiological sciences are better comprehended as multi-system interaction. The interplay of several signals released by different tissues may greatly affect distal organs function. This can occur under a healthy state but mainly under pathological states. There are several examples that may reinforce those interactions. For instance, after brain injury, distal organs may suffer from decreased function that is not entirely explained by others causes. Recently, we faced and are still facing the consequences of COVID-19. In this disease, it is evident the damage of different organs, among them brain, lungs, kidneys, gut, which may contribute to high rate of morbidity and mortality. During this symposium, the researches will join all the evidence in their respective research fields in order to demonstrate that the study of physiological sciences are integrative among different organs instead of independent views of specific organs. The present talk will focuss on the cross-communication between lungs and brain.

S5-2

Cross talk between brain and heart

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The symposium theme, "Cross Talks between different organs," aims to explore intriguing instances of interaction between physiological systems. It delves into research that occurs exclusively at the intersections of different organs and systems. Within the Brain-Heart section, we have selected a segment of our research that centers around SUDEP (Sudden Death in Epilepsy). Our work involves employing a Severe Scorpion Envenomation (SSE) animal model to induce epileptiform activity and seizures. Notably, this process leads to various physiological disruptions, including heart arrhythmias, AV blockage, pulmonary edema, and ultimately, death.

Our research findings indicate that these peripheral systems may be compromised primarily as a consequence of central nervous system effects, with no direct action of the toxin on the heart or lungs. In fact, we have observed that drugs that modulate neuronal activity in central areas, such as carbamazepine or phenobarbital, can effectively reverse the catastrophic effects of SSE on the heart and lungs. This data not only suggests that SUDEP, in the context of our particular model, may result from brainstem recruitment during seizures, but it also introduces a novel therapeutic strategy. This strategy, which complements antiscorpion venom serum treatment, involves safeguarding the brainstem from hypersynchronous activity.

Financing: CNPq - FAPEMIG - FABLAB Lemann Foundation

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S5-3

Cross talk between kidney and other organs

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The objective of this study is to utilize zebrafish larvae to determine if increased fructose intake can induce renal injury and whether this injury correlates with the production of reactive oxygen species. We also seek to elucidate the regulatory mechanisms contributing to AKI associated with the diet or crosstalk with the liver. **Methods:** To investigate these associations, we employed the *Danio rerio* model, which shares renal anatomical similarities with humans and serves as an established model for studying AKI and NAFLD. Zebrafish larvae were exposed to diets mimicking the Western Diet and assessed for inflammatory

markers, renal injury, and immune cell migration patterns, particularly neutrophils. **Results:** Our investigations revealed that the Western Diet indeed triggered renal and hepatic injuries, accompanied by increased systemic inflammation and alterations in neutrophil migration dynamics. Using microscopy, we observed a change in the dynamic movement of neutrophils due to the diet. However, the absence of neutrophils did not alter the profile of AKI at the observed stage of the disease. Our findings provide insights into the regulatory mechanisms contributing to AKI and communication with the liver.
Financing: FAPESP 2017/05264-7CAPES, financial code 001CNPq

S6. NEW HORIZONS IN CARDIORENAL ION TRANSPORT

Chairs: Oleg Palygin (Medical University of South Carolina, USA), Daria Ilatovskaya (Augusta University, Medical College of Georgia, USA)

S6-1

Mitochondrial calcium uniporter complex and its physiological and pathological roles in the heart

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Mitochondrial calcium ($mtCa^{2+}$) entry controls numerous cell functions, including energy metabolism, reactive oxygen species (ROS) generation, spatiotemporal dynamics of Ca^{2+} signaling, cell growth/development and death in the heart. Since the discovery of the molecular identity of the $mtCa^{2+}$ uniporter protein complex (mtCUC), over the last several years, multiple groups, including ours, have taken advantage of newly available information about mtCUC and applied genetic tools to delineate the role of $mtCa^{2+}$ uptake in the heart, especially focusing on the regulation of mtCUC in the cardiomyocytes, a main cell-type in the heart. It has been well recognized that $mtCa^{2+}$ overload via mtCUC activation is one of the key determinants of ROS generation and cellular damage in the ischemic heart disease. However, it is still not clear whether $mtCa^{2+}$ uptake can also impact the functions of cardiac fibroblasts, another major cell-type in the heart. In this presentation, we introduce our recent finding of the molecular mechanism of how mtCUC-mediate $mtCa^{2+}$ uptake promotes proliferation of cardiac fibroblasts and cardiac fibrosis in heart. We will also emphasize the alteration of Ca^{2+} handling at the endoplasmic reticulum (ER)-mitochondrial (ER-Mito) microdomains during cardiac stress, and how this may alter $mtCa^{2+}$ and ROS levels in the cardiac fibroblasts under cardiac pathology.
Financing: NIH R01HL136757

S6-2

Ghrelin enhances tubular magnesium absorption in the kidney.

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Animal models mimicking bariatric procedures display bone disease, decreased serum Ca^{2+}, Mg^{2+} and the gastric hormone Ghrelin. Ghrelin binds to the growth hormone secretagogue-receptor (GHSR) which is expressed in renal tubules. We tested if Ghrelin modifies tubular calcium or magnesium absorption via the ion channels TRPV5 or TRPM6.

After Ghrelin exposure whole-cell current density did not change for TRPV5 but increased for TRPM6. While a Ghrelin-mimetic also increased TRPM6 current density, addition of a GHSR antagonist inhibited the effect. GHSR signals via protein kinase A (PKA) and applying the PKA inhibitor H89 abrogated TRPM6 stimulation by Ghrelin. In microdissected tubules of wild-type (WT) mice there was Ghrelin and GHSR mRNA in the TAL with 50% lower levels in microdissected DCTs. TRPM6 was highly expressed in the DCT. We also detected TRPM6 mRNA in the TAL at 15% expression compared to DCT. Immunofluorescent studies of GHSR-GFP mice confirmed a GFP signal in the TAL but not in the DCT. In GHSR-null and WT mice, baseline serum magnesium and 24-hour urinary excretion of magnesium was not significantly different. Starved GHSR-null mice displayed a significantly higher urinary magnesium excretion and lower serum magnesium levels with downregulation of tubular magnesiotropic genes.
Given the higher GHSR mRNA abundance in the TAL compared to DCT the significance of Ghrelin

stimulating TRPM6 via GHSR and $G\alpha_s$-PKA signaling remains unclear. Higher urinary magnesium excretion in starved GHSR-null mice point to Ghrelin-upregulation of TRPM6 in the TAL and/or upregulation of other magnesiotropic genes.

Financing: Department of Defense (W81XWH1910205), the National Institute of Health (R01DK119631, DK079328-11), and the Children's Clinical Research Advisory Committee (CCRAC), Children's Health System, Dallas

S6-3

Water is life: defending against dehydration

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The need to maintain adequate body water has posed a challenge to terrestrial organisms since their emergence from the sea. High solute consumption, such as a high salt diet, poses a risk for osmotic diuresis and dehydration. We are studying mechanisms by which organisms sense and respond to high salt osmotic stress using the fruit fly, *Drosophila melanogaster*. I will present data obtained from the study of wild-type flies and two mutants. Flies carrying a mutation in the chloride channel encoded by *bestrophin-1* have increased lethality on high salt diet that is due to a failure to upregulate hemolymph (plasma) osmolytes. In contrast, flies carrying a mutation in a regulator of G protein signaling, encoded by *locomotion defective (loco)*, have decreased lethality on high salt diet. *Loco* mutants better conserve water on high salt and have an altered metabolic profile, which may underlie their ability to cope with high salt stress. Prior studies in rodents suggest these studies are relevant to mammalian adaptations to high salt osmotic stress.

Financing: National Institutes of Health, DK110358

S6-4

Protease activated receptors and glomerular function

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Mesangial cells provide structural support to the glomerular tuft and modulate the glomerular capillary flow via their contractile properties. Mesangial cells' phenotypic changes into myofibroblast-like cells are associated with cell proliferation, mesangial expansion, abnormal glomerular tuft formation, and reduced numbers of capillary loops, which are present in several glomerular diseases, including diabetic nephropathy and glomerulonephritis. In addition, thrombin-induced mesangial remodeling was found in diabetic patients, and expression of the corresponding protease-activated receptors (PARs) in the renal mesangium was reported. However, the functional PAR-mediated signaling and mechanisms in mesangial cells were not examined. This study aims to investigate protease-activated mechanisms regulating mesangial cell contraction and glomerular capillary flow. Our results indicate that coagulation proteases like thrombin may strongly regulate mesangial cell contraction and corresponding glomerular capillary flow by PAR1 GPCRs-related activation. The contraction mechanism is mediated presumably through SOCs entry and TRPC3 channels. Since high thrombin levels are linked to poor diabetic control, the described signaling may play a crucial role in the development of glomerular pathology and diabetic nephropathy complications.

Financing: R01 NIDDK DK126720 (to OP) and DK129227 (to AS and OP)

S7. NOVEL ASPECTS OF CELL COMMUNICATION IN THE MICROCIRCULATION

Session in Honor of Professor Walter Durán
Chairs: Mauricio Boric (Pontificia Universidad Católica de Chile, Chile), Daniel González-Reinoso (Universidad de Talca, Chile)

S7-1

Limiting Microvascular Hyperpermeability in the Injured Host

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Microvascular hyperpermeability is a significant clinical problem leading to edema, poor tissue oxygenation, and tissue/organ dysfunction. The endothelial cells of the postcapillary venules play an active role in controlling leakage of plasma components but their function becomes impaired in response to inflammation caused by disease or injury. Subcellular structures including the cytoskeleton, intercellular junctional protein complexes, focal adhesion complexes, and the glycocalyx surface layer all play key roles in endothelial barrier function. In recent years, our focus has been on finding ways to limit microvascular permeability in response to hemorrhagic shock and other inflammatory insults. This talk will discuss the potential of using sphingosine-1-phosphate (S1P) to protect the endothelium, and will highlight recent data from our laboratory highlighting subcellular mechanisms involved in restoring endothelial barrier function.

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S7-2

eNOS signaling via S-nitrosylation in leukocyte and tumor cell adhesion

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A key modulator of the endothelial function is nitric oxide (NO). We have reported that the stimulation of endothelial cells with secreted factors from breast tumor cells (TNF-a, IL-8, kallikrein-related peptidases) induce S-nitrosylation (the modification by NO of cysteine residues in proteins) of endothelial proteins leading to destabilization of the endothelial barrier which may contribute to transmigration of breast tumor cells and metastasis. The attachment of cancer cells to the endothelium is the first step in the extravasation process leading to metastasis. This step shares similarities with leukocyte adhesion to the endothelium, and it is plausible that it may also share some regulatory elements. Here we report that the stimulation of endothelial cells with cytokines present in the serum of cancer patients (TNF-a, IL-8) or with secreted factors from breast tumor cells activates the S-nitrosylation pathway and increases leukocyte adhesion in vitro and in vivo. The stimulation also increases the cell surface availability of the adhesion proteins VCAM-1 and ICAM-1 in endothelial cells in a NO and S-nitrosylation dependent way. We identified PKCz and VCAM-1 as S-nitrosylated targets during this process. Inhibition of NO signaling and S-nitrosylation also blocks the transmigration of tumor cells through endothelial monolayers and the development of metastasis in a murine model of breast cancer. We propose that S-nitrosylation in the endothelium activates pathways that enhance surface localization of adhesion proteins to promote binding of tumor cells and extravasation leading to metastasis.

Financing: Fondecyt1201635

S7-3

Connexins and Pannexins in the Regulation of Vascular Tone

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Introduction: Connexin-formed channels (gap junctions and hemichannels) play a central role in the endothelium-dependent coordination of vasomotor tone in resistance arteries by the transmission of electrical signals and nitric oxide (NO). However, the participation of Panx-1-formed channels in the control of endothelial cells function has not been determined.

Objective: To evaluate whether Panx-1 channels are involved in the control of vasomotor tone in resistance arteries.

Methods: Changes in NO-mediated vasodilation, membrane potential, superoxide anion ($O_2^{\cdot -}$) and endothelial cell $[Ca^{2+}]_i$ were analyzed in rat isolated mesenteric arterial beds and primary cultures of mesenteric endothelial cells. All studies were approved by the Institutional Bioethics Committee (Protocol #210422002). Data were analyzed by Student's paired t-test or by two-way ANOVA.

Results: The endothelium-dependent vasodilation induced by acetylcholine (ACh, 100nM) and the phosphorylation of endothelial NO synthase (eNOS) at serine 1177 (P-eNOS^{S1177}) and Akt at serine 473 (P-Akt^{S473}) were enhanced after Panx-1 channel inhibition with probenecid or ^{10}Panx, which was associated with a tetrodotoxin (300nM) sensitive depolarization and an $[Ca^{2+}]_i$ increase in endothelial cells. The endothelial cell depolarization was converted into a transient spike in the presence of 10µM Ni^{2+} or 100µM mibefradil. Application of Ni^{2+} also abolished the increment in $[Ca^{2+}]_i$. Furthermore, Panx-1 channel blockade leads to an increase in $O_2^{\cdot -}$ production. TEMPOL or apocynin prevented the increase in $O_2^{\cdot -}$, ACh-induced vasodilation, P-eNOS^{S1177} and P-Akt^{S473} observed in response to Panx-1 inhibition.

Conclusions: Panx-1 channels regulate NO-mediated vasodilation through a novel signaling pathway initiated by the sequential activation of Na_V and $Ca_V3.2$ channels.

Financing: Grant Anillo ANID/ACT210057

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S7-4

Potential use of circulating microalgae for photosynthetic tissue oxygenation. Systemic and ex vivo approaches.

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Animal cells cannot produce oxygen for aerobic metabolism; thus, they have an extreme dependency on microvascular oxygen delivery by flowing erythrocytes. Instead, several small cells produce oxygen by photosynthesis, posing the question of whether they could circulate within the vascular networks, acting as an alternative source for oxygen delivery upon illumination. This idea was addressed using a cell-wall deficient photosynthetic microalga *Chlamydomonas reinhardtii (C.r.)* as model organism. This microalga grows and survives in standard extracellular mammalian media, sharing size and rheological properties with erythrocytes. Also *C.r.* can be co-cultured with endothelial cells, without affecting each other's morphology and viability; and the systemic injection of high numbers of microalgae did not trigger immune or deleterious responses in living mice. Short-term systemic perfusion of *C.r.* in mice showed a thorough intravascular distribution up to capillary networks. To explore whether intravascular photosynthesis could prevent hypoxia *ex vivo*, *C.r.* was incorporated in a standard organ preservation solution and used to perfuse rat and porcine kidneys. Microalgae reached all renal vasculature, without inducing tissue damage. After flushing, recovered microalgae survived the process. When illuminated *in vitro*, this *C.r.* solution can fulfill the metabolic oxygen demand of rat kidney slices; moreover, rat kidney slices obtained from *C.r.* perfused organs exhibited significantly improved preservation after 24 hours of incubation in hypoxia and light, resulting in reduced tissue injury and enhanced metabolic status. Consequently, intravascular photosynthesis represents a promising approach for tissue oxygenation *ex vivo* that could be further applied to preserve organs for transplantation purposes.

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S8. LIPID METABOLISM AND ADIPOSE TISSUE IN EATING BEHAVIOR AND METABOLIC REGULATION

Sponsored by Elsevier

Chairs: René Braudand (Pontificia Universidad Católica de Chile, Chile), José Galgani (Pontificia Universidad Católica de Chile, Chile)

S8-1

Risk factors for adiposopathy across the lifespan

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The expansion and remodeling of adipose tissue during obesity is an adaptive process that buffers excess energy and protects against lipid spillover into the circulation. Adipose expansion is driven by 1) hypertrophy of existing adipocytes and 2) de novo generation of adipocytes from a resident pool of adipocyte progenitor cells (APCs). Transition from metabolically healthy to unhealthy obesity is triggered by adiposopathy or "sick fat" due to a failure in the recruitment of APCs to support high demands for lipid storage. Late fetal life is a critical window of lineage commitment that establishes two compartments of APCs: a developmental pool that gives rise to adipocytes during postnatal establishment of adipose depots and APCs that reside in adult depots to support adipose remodeling. Therefore, childhood adiposity and adipose plasticity in adulthood are uniquely regulated by distinct APC populations and may both be influenced by the intrauterine environment. This talk will highlight our recent work demonstrating that maternal obesity during pregnancy accelerates developmental adipogenesis in the offspring, thereby predisposing to later-life obesity and metabolic syndrome. Further, we will discuss sex differences in adipose plasticity that arise after puberty and show our recent data implicating estrogen in mediating sex differences in APC responses to obesity.

Financing: Canadian Institutes of Health Research (CIHR), National Sciences and Engineering Research Council of Canada (NSERC), Heart and Stroke Foundation of Canada

S8-2

Determinants of insulin resistance-associated fatty liver disease

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Fatty liver disease is the hepatic manifestation of insulin resistance syndrome. Hepatic steatosis results from the disequilibrium between the mechanisms that promote fatty acid accumulation (extracellular uptake and de novo lipogenesis) and those that promote its hepatic clearance (exportation into lipoproteins and oxidation). Nevertheless, the most relevant processes are those that determine the inflammatory, fibrotic, and oncogenic responses to lipid overload because they determine cirrhosis and hepatocarcinoma. We have studied fatty liver disease in patients and murine models of obesity and lipodystrophy to understand the mechanisms underlying both steatosis and progression. In the lipodystrophic AGPAT2 deficient mice we found that the main mechanism of steatosis is exaggerated de novo lipogenesis depending on the transcriptional control of ChREBP and not SREBP1c. In addition, we have found that leptin completely reverses fatty liver in lipodystrophic mice by mechanisms independent of the hepatic leptin receptor. Finally, the gene deletion of insulin sensitizing Fibroblast growth factor 21 increases liver triglycerides in both, lipodystrophic and obese leptin receptor deficient mice. In obese patients we found that protein and mRNA levels of the inflammasome NRLP3 system do not correlate with histological nor systemic markers of hepatic inflammation and insulin resistance. More recently, interorganelle contacts between lipid droplets and mitochondria have risen as potential mechanisms to modulate hepatocellular fatty acid metabolism to prevent lipotoxicity.

Financing: Fondecyt 1221146 Anillos ACT 210039

S8-3

Diet induces neural plasticity-associated modifications and epigenetic changes in the hypothalamus

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The prevalence of obesity has nearly tripled in the last 40 years, suggesting the contribution of environmental factors associated with the modern lifestyle. Hypothalamic neuronal circuits control energy homeostasis and maintain high levels of plasticity through the permanent control of gene expression, a process in which epigenetic factors play a pivotal role. However, the influence of diet composition on epigenetic modifications and its impact on hypothalamic neuronal plasticity has only been recently addressed.

We aimed to evaluate epigenetic changes underlying the hypothalamic neural plasticity-related modifications associated with high-fat/low-carb (HFLC) feeding. We used WT and transgenic mice fed since weaning with an HFLC diet for 4 or 12 weeks (CEC-USS 14-2020-10).

We observed that mice fed an HFLC diet exhibited changes in body weight, food intake, and energy expenditure. Besides, these changes were associated with both modifications in the cytoarchitecture of hypothalamic neurons and gene expression patterns. We also found changes in the expression/phosphorylation of the epigenetic reader Mecp2 and miRNAs expression, which could underlie the changes in the neuronal cytoarchitecture and gene expression associated with sensitivity to metabolic signals involved in neuroendocrine integration and maintenance of energy balance.

Our results show that changes in energy demand alter miRNAs and protein expression, commanding chromatin remodeling and modifying the hypothalamic expression of neuronal plasticity-related genes, which impacts the hypothalamic neuronal cytoarchitecture, feeding behavior, and energy expenditure. Our results highlight the role of chromatin remodeling in the proper hypothalamic function required for adequate control of neuroendocrine integration and energy balance

Financing: Fondecyt 1180905 1230905; ANID-Anillo ACT210039

S8-4

Insights into feeding behaviour in different environments: From animal models to humans

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Animals and humans evolved behaviors to find and select high-calorie food in an uncertain environment where energy-dense foods were seldom available. However, the modern environment makes readily available palatable food (rich in fat and sugar) and causes hedonic intake, which is to experience pleasure despite satiety and causes weight gain and obesity. We aim to understand the neuroendocrine control of feeding behavior under different environments and its relationship with obesity by focusing on different neuropeptides, including GLP1. GLP1 is

an anorectic peptide that acts in central and peripheral tissues to reduce intake, induce lipolysis in white adipose tissue (WAT), and reduce glycemia. We showed that the anorectic effects of GLP1 depend on the availability of preferred foods within an obesogenic environment and that early exposure to an obesogenic environment can alter the effects of GLP1 on food intake and WAT during the early stages of obesity development. Further, data suggest that the action of GLP1 to regulate intake is dependent on the action of leptin, another hormone key for the maintenance of energy balance. We are expanding our studies towards humans by analyzing conflict during food choice, and by analyzing whether context, sex, obese state, and nutritional information alter the choice of healthy foods in computer-based trials. Together, these studies aim to expand our understanding of how neuronal mechanisms controlling food intake are altered in obesity.

Financing: ANILLO GRANT ACT210039, FONDECYT REGULAR 1200578

S9. HOT TOPICS IN CHRONIC KIDNEY DISEASE

Chairs: Timo Rieg (University of South Florida, USA), Jessica Dominguez (University of South Florida, USA)

S9-1

Intracellular Calcium Signaling in Podocytes in Diabetic Nephropathy

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Diabetic nephropathy (DN) is a leading cause of chronic kidney disease (CKD), where the kidneys fail to function properly. One of the main causes of DN is podocyte injury. Podocytes have limited proliferative capacity, and when glomerular growth and hemodynamic stresses exceed the ability of podocytes to undergo hypertrophy, they become irreversibly injured. It has been documented that Ca^{2+} signaling in podocytes plays a pivotal role in glomeruli function. High glucose levels and other factors associated with diabetes can lead to injury and dysfunction of podocytes. The exact mechanisms by which this occurs are complex and not fully understood. Here, I will provide some mechanistic insights into the mechanisms of elevated intracellular calcium in podocytes under diseased conditions. Specifically, I will uncover several potential mechanisms, such as the activation of protease-activated receptors (PAR) or cyclic GMP-AMP Synthase (cGAS) / STimulator of INterferon Genes (STING) signaling pathway, which lead to the progression of CKD. Furthermore, the type 2 diabetic nephropathy (T2DN) rats, as a rodent model to study DN, will be introduced. T2DN rats were previously created by introgression of the mitochondria and some passenger loci from the Fawn Hooded Hypertensive rat onto the background of the Goto-Kakizaki rat. We have demonstrated that T2DN rats develop renal and physiological abnormalities similar to clinical observations in humans with DN, including progressive glomerular damage and a significant decrease in renin-angiotensin-aldosterone system plasma levels, indicating these rats are an excellent model for studying the progression of renal injury in type 2 DN.

Financing: The research in the laboratory was supported by the National Institutes of Health grants R01 DK135644 and R01 DK129227, and the Department of Veteran Affairs grant I01 BX004024.

S9-2

The role of Atrial Natriuretic Peptide signaling in kidney disease progression

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There is no specific treatment available for patients with salt sensitivity of blood pressure (BP); unfortunately, the molecular mechanisms underlying salt-sensitivity remain poorly understood. One of the major proposed mechanisms for the development of salt-sensitive hypertension (SSH) involves a defect in the ability of the kidneys to excrete salt. Atrial Natriuretic Peptide (ANP) encoded by Nppa, is a hormone known to promote salt excretion and BP reduction, and there are clinical data implicating inherently low levels of ANP in the development of SS hypertension. Among other effects, ANP (via cGMP-related mechanisms) is known to be

beneficial for mitochondrial bioenergetics and biogenesis, as shown in studies on heart and fat tissues. However, there is a gap in knowledge regarding the effects of ANP on mitochondria in the kidney, especially in SS hypertension. In our recent studies, we revealed distinct metabolic and bioenergetics profiles of kidney cortex in Nppa knockout compared to wild type Dahl SS (Salt-Sensitive) rats, which are differentially affected by a high salt diet. A combination of in vivo techniques with studies performed on isolated renal mitochondria and renal metabolomics are employed to establish the distinct renal mitochondrial bioenergetic profiles contributing to the development of SSH. The successful completion of the ongoing studies will unravel the novel causative mechanisms of salt-sensitivity.

Financing: NIH R01 HL148114 and U54HL169191

S9-3

Development and Application of a Nanoparticle Delivery System that Selectively Targets Kidney Glomeruli

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Collagen IV (col4) expressed in the glomerular basement membrane (GBM) is the only area directly contacting with blood via fenestrated capillary endothelium. Liposomes have been extensively used as drug carriers due to their excellent biocompatibility and low immunogenicity. Herein, we formulated a novel col4-targeted nanoparticle (Col4-NP) by coupling liposomes with a col4-targeted peptide, which selectively targets glomeruli by binding to col4 in GBM.

We found that rhodamine-labeled Col4-NP mainly accumulated to the kidney glomeruli 24 hours post i.v. injection. Col4-NP system exhibited stable and sustained release of the loaded content over 3 days. Lupus-prone mice treated with prednisolone-loaded Col4-NP exhibited significant improvement in kidney functions reflected by 30% higher in glomerular filtration rate and 56% less proteinuria and histological structures with reduced IgG deposition. The treatment with prednisolone-loaded Col4-NP increased renal Tregs in the lupus mice.

We believe that the development of the glomerulus-targeted therapeutic nanoparticles has substantial translational significance and may offer potent site-specific treatments for lupus nephritis patients with minimized systemic side effects. These glomeruli-targeted NPs may serve as a glomeruli-specific delivery system to achieve precise therapeutics for different kinds of glomerular diseases.

Financing: DK134000, DK134028, HL142814

S9-4

Iron Deficiency Anemia: Microbiome Changes in Response to Intravenous Iron Administration

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Iron deficiency anemia (IDA) is a major global health issue that affects 30% of the population. Replenishing iron stores with either oral or intravenous (IV) supplementation is the mainstay of IDA treatment. There is a complex bidirectional interplay between the host's iron status, gut microbiota, and dietary iron availability. Dietary iron deficiency and supplementation can influence the gut microbiome; however, the effect of IV iron on the gut microbiome is unknown. We investigated the effects of the IV iron preparations ferric carboxymaltose (FCM) and ferric derisomaltose (FDI) on the gut microbiome in female iron-deficient anemic mice. Vehicle-treated mice exhibited an expansion in Verrucomicrobia, primarily due to the increased abundance of *Akkermansia muciniphila*, as well as contraction in *Firmicutes*, resulting in a lower *Firmicutes/Bacteroidetes* ratio (indicator of dysbiosis). Treatment with either FCM or FDI restored the microbiome such that *Firmicutes* and *Bacteroidetes* were the dominant phyla.

Interestingly, when comparing mice treated with FCM to those treated with FDI, the phyla *Proteobacteria* and several members of *Bacteroidetes* (e.g., *Alistipes*) were expanded. In contrast, several *Clostridia* class members were expanded in mice treated with FDI compared with FCM (e.g., *Dorea spp*., *Eubacterium*). Our findings show that IV iron increases gut microbiome diversity independently of the iron preparation used; however, differences exist between FCM and FDI treatments. In conclusion, replenishing iron stores with IV iron preparations in clinical conditions, such as inflammatory bowel disease or chronic kidney disease, may change the composition of the gut microbiome and consequently contribute to an altered disease outcome.

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S10. PHYSIOLOGICAL AND MOLECULAR REGULATION OF THE HYPOTHALAMIC-NEUROHYPOPHYSIAL SYSTEM

Chairs: André Mecawi (Federal University of São Paulo, Brazil), David Murphy (University of Bristol, UK)

S10-1

Multi-omics analyses of the hypothalamic-neurohypophysial system

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Hypothalamic magnocellular neurons, primarily located in the supraoptic and paraventricular nuclei, play a pivotal role in producing and releasing the neuropeptides vasopressin (AVP) and oxytocin (OXT) into the bloodstream through the neurohypophysis. These neuropeptides are vital regulators of renal water reabsorption and female reproductive function. Recent research has shed light on the multifaceted roles of hypothalamic magnocellular neurons. It is now evident that these neurons not only release neuropeptides into the bloodstream but also project collateral axons to various brain regions, influencing a range of bodily, emotional, and cognitive functions. Additionally, it appears that hypothalamic magnocellular neurons are more diverse than previously thought, extending beyond just two phenotypes responsible for producing AVP or OXT. Therefore, comprehending the molecular regulation and transcriptional diversity of hypothalamic magnocellular neurons is imperative for unraveling the intricate regulatory functions they govern. This presentation will delve into the recent multi-omic data that describe the spatial transcriptome, proteome, phosphoproteome, and lipidome data from the hypothalamic supraoptic nucleus (neuronal bodies) and the neurohypophysis (axon terminals). This data will be integrated into a newly created single-cell RNA sequencing atlas from the rat hypothalamic supraoptic nucleus to provide a comprehensive insight into the molecular diversity of magnocellular neurons and their responses to physiological stimulation induced by water deprivation.

Financing: FAPESP: #2019/27581-0

S10-2

Astrocytic modulation of the hypothalamic magnocellular neuron's activity in the supraoptic nucleus

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One remarkable aspect of neural networks is their capacity to swiftly adapt structurally and functionally when faced with challenges, ensuring the preservation of their functionality. In the supraoptic nucleus (SON), which is one of the nuclei involved in responding to hydroelectrolytic disorders, it is well-documented that significant structural changes lead to alterations in the morphology, activity, and function of the cells within this nucleus. However, the electrical

implications of such changes remain unexplored, particularly in the context of astrocyte-magnocellular neuron communication. In this regard, we sought to understand whether glial cells, specifically astrocytes, influence the electrical properties of magnocellular neurons during short-term acute hypertonicity. To do so, we combined single and double whole-cell patch-clamp recordings and utilized genetically modified animals to understand the role of astrocytes on the excitability of magnocellular neurons when faced with increased plasma osmolality. Our findings revealed that astrocytes are themselves responsive to short-term hypertonic stimulation. Notably, double-patch recordings (involving both neurons and astrocytes) indicated that the depolarization of the resting membrane potential in astrocytes precedes the heightened activity of magnocellular SON neurons. Additionally, we observed calcium oscillations in SON astrocytes during hypertonic stimuli, suggesting a potential mechanism for the membrane depolarization of these cells. In conclusion, the results obtained thus far provide valuable insights into the contribution of astrocytes to the regulation of magnocellular neuron excitability during hypertonic conditions, as well as the synaptic and biophysical mechanisms underlying this intricate process.
Financing: FAPESP, CAPES

S10-3

Sex differences in the neurohypophyseal system in an animal model of cirrhosis

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Dilutional hyponatremia is known to increase morbidity in patients with advanced liver disease due to the inappropriate release of arginine vasopressin (AVP). Our previous studies show that bile duct ligation (BDL), an animal model of cirrhosis, is associated with decreased plasma osmolality but increases circulating copeptin in male rats. Female BDL rats do not show increases in circulating copeptin or decreases in plasma osmolality as compared to sham females. Instead, female BDL rats show increases in circulating oxytocin. We conducted electrophysiology experiments to determine if changes in chloride regulation occur in AVP cells from the supraotpic nucleus (SON) in male BDL rats but not females. Cells from male BDL rats showed impaired $GABA_A$-mediated inhibition in response to muscimol, as compared to those from male sham rats. All putative AVP SON neurons from sham rats (4/4) showed decreased activity following muscimol while half of the putative AVP SON neurons (7/14) from male BDL rats were excited ($p<0.001$). Similar results were observed in experiments where the injections missed the SON. In these experiments, all SON neurons from sham rats were inhibited by muscimol (26/26) while thirty-five percent of SON neurons from male BDL rats (16/40) were excited by muscimol ($p = 0.001$). In experiments with cells from female BDL rats, 81% (13/16) of putative AVP cells were inhibited by muscimol. The results show that liver cirrhosis impairs the normal inhibitory response of AVP SON neurons to $GABA_A$ receptor activation which could contribute to increased cell activity and dilutional hyponatremia in male rats.
Financing: Supported by R01 HL142341

S10-4

Effect of early programming stimuli on magnocellular neurons and their osmoregulatory responses

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Abstract: The obligatory increase in sodium intake during the perinatal period has programming effects that affect cardiovascular function and the vasopressinergic system, causing anatomical and molecular changes at the renal, cerebral, and vascular levels culminating in an increase in blood pressure in the progeny. However, the effect of perinatal natriophilia on blood pressure control mechanisms and the vasopressinergic system is not known. To this end, we evaluated the effect of voluntary hypertonic sodium intake during the

perinatal stage on blood pressure control and gene expression at the renal and brain levels after a sodium overload (SO) challenge in adult offspring (PM-NaCl group). Experiments were approved by the ethics committee (#009/2019) and results were analyzed with two-way ANOVA and post hoc tests. A sustained increase in blood pressure was observed after SO in male animals relative to their controls (PM-Control). In the supraoptic nucleus, SO did not modify the relative expression of AVP (precursor and neuropeptide) in PM-NaCl in contrast to the increase observed in controls. At the renal level, PM-NaCl animals showed a reduction in the number of glomeruli, a decrease in transient receptor potential vanilloid receptor type 1 mRNA expression, and an increase in angiotensinergic receptor type 1 mRNA expression, with no change in vasopressinergic receptor 2 in the renal cortex compared to PM-Control. These results suggest that the availability of a sodium-rich resource during the perinatal stage has a long-term programming effect on neuroendocrine, renal, and cardiovascular responses induced by osmotic challenges, impacting the regulation of homeostasis.

Financing: CONICET, FONCyT and SECyT

S11. MUSCLE-ORGAN CROSSTALK: FOCUS ON DISEASES

Chairs: Paola Llanos (Universidad de Chile, Chile), Denisse Valladares (Universidad de O´Higgins, Chile)

S11-1

Molecular linkers between skeletal muscle atrophy and bone loss after muscle paralysis

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Muscles and bones are coordinately remodeled according to demand in health and disease. In addition to mechanical communication, there is biochemical communication between both tissues. We have previously shown that extracellular ATP (eATP) is a molecule released from skeletal muscle during contraction and regulates the expression of myokines, such as interleukin-6 (IL-6), that improve exercise performance. However, aged or pathological muscles (dystrophic, denervated) have overactivated the eATP pathway. In addition, it is known that the persistent increase in plasma IL-6 participates in muscle and bone damage in several chronic pathologies. Then, we have focused on the role of eATP/IL-6 pathway in musculoskeletal damage generated by hypofunction.

We particularly studied the masticatory system, highly remodeled throughout life. Two models of masticatory muscle atrophy were addressed in adult mice (8 weeks-old): soft-diet feeding, or unilateral injection of Botulinum Toxin Type A (BoNTA) in masseter muscle (IACUC-UChile #CBE-FOUCH061501/#20381-ODO-UCH). Muscles and bones were addressed by micro-computed tomography (µCT) and 3D-analysis, histology, RT-qPCR and immunoblot.

Muscle paralysis or hypofunction led to muscle atrophy and bone loss. The eATP pathway was overexpressed in atrophied muscles. Increased levels of eATP, P2Y/P2X ATP receptors, and ATP-releaser conduits (pannexin, connexin) were observed. Atrophied muscles raise the mRNA and protein expression of IL-6 and its receptors (IL-6R, gp130). Interestingly, atrophied muscles were less sensitive to exogenous ATP, raising the possibility of a deregulation in ATP signaling.

We are currently addressing whether the deregulation in the eATP/IL6 pathway directly participates in the induction of muscle atrophy and bone loss.

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S11-2

Gut-Muscle-Brain axis: The role of gut microbiota on muscle and cognitive function during old age.

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Introduction: Sarcopenia and cognitive decline in older adults are linked to gut microbiota changes. Exercise can improve gut microbiota, offering potential benefits for healthy aging and

preventing age-related declines in muscle and cognitive functions.

Objective: Fecal microbiota transplants (FMT) from young mice donors, submitted to physical training, to older mice will restore the microbial diversity in the recipient's gut microbiota, improving the muscle and cognitive function.

Methods: C57BL/6 male mice at 12 and 18 months old were evaluated before and after receiving FMT from young and trained mice (4 months old). The assessment included muscle function, cognitive performance, and electrophysiological recordings. Data are presented as mean ± SEM (n=8), and Mann-Whitney test for statistical comparisons. Bioethics approval from Universidad de Valparaíso CICUAL code BEA179-22.

Results: 16S rRNA sequencing revealed more diverse fecal microbiota in trained mice compared to sedentary ones, with distinct bacterial family representation in aged mice receiving FMT from young-trained donors. FMT from trained mice enhanced muscle strength in middle-aged and aged mice, evaluated through weight lifting and gripping tests. Cognitive function improved in both age groups with FMT from trained mice, assessed via novel object recognition and object location memory tests. Electrophysiological recordings showed treated animals regained the ability to generate long-term potentiation (LTP), associated with synaptic plasticity and memory formation, which was reduced in control aged mice.

Conclusion: Enhancing gut diversity through FMT can benefit muscle and cognitive function in older individuals, offering a promising solution for conditions like sarcopenia and cognitive decline in older adults.

Financing: Project funded by FONDECYT 1122097 and NAM21I0063.

S11-3

Exercise regulation of hepatic LD-mitochondria interaction in non-alcoholic fatty liver disease

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Lipid Droplets (LDs) are highly dynamic storage organelles. In the liver, its accumulation causes non-alcoholic fatty liver (NAFL) that can progress to a more severe disease stage, non-alcoholic steatohepatitis (NASH). LDs interact with mitochondria, which impacts lipid homeostasis. However, whether exercise modulates this interaction in the liver has not been studied.

To examine the effects of aerobic exercise on the liver, two different NAFLD models were used: a high-fat diet (HFD) to evaluate NAFL and an HFD-methionine choline-deficient diet (MCD) to evaluate NASH. Our results in the NAFL model showed that exercise decreased disease severity and improved physical capacity compared to sedentary HFD mice. Although exercise increased the number of LDs in hepatocytes, LDs were smaller than in the sedentary HFD mice. Notably, while sedentary HFD mice had increased hepatic lipid droplet (LD)-mitochondria interaction, in exercised HFD mice, there was a decreased interaction. The findings of the NAFL model are consistent with those of the NASH model. Aerobic exercise increased fatty acid oxidation and Mitofusin-2 abundance in peridroplet mitochondria. Strikingly, the absence of Mitofusin-2 (Mfn-2 LKO mice) prevents the exercise-induced fatty acid b-oxidation of PDM. Taken together, our findings show that aerobic exercise reduced the progression of NAFLD by promoting reduced lipid droplet-mitochondria interaction in hepatocytes and enrichment of Mitofusin 2 in peridroplet mitochondria, increasing fatty acid β-oxidation.

Financing: FONDECYT 1191078, ENLACE FONDECYT 01/23

S11-4

Connecting the dots: how muscle-resident fibro-adipogenic precursors and adipocytes support muscle tissue metabolic flexibility

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The ability of skeletal muscle tissue to adapt myofiber ATP production to the interstitial availability of energy fuels, i.e., metabolic flexibility, assures an adequate energy supply to sustain the metabolic demands of contractile activity. Fibro-adipogenic progenitors (FAPs) are

muscle-resident mesenchymal stromal cells that play a critical role in providing a proper cellular niche for the maintenance of muscle mass homeostasis. Thus, FAPs can adapt to metabolic challenges in skeletal muscle by remodeling their metabolic machinery in accordance with the prevailing metabolic signals and available energy substrates in surrounding cellular environment. In homeostatic conditions, FAPs mainly rely on mitochondrial fatty acid oxidation for energy production. Conversely, studies have described a metabolic switch in FAPs exposed to pathological conditions, which can drive adipogenic differentiation of these cells. The aim of this talk is to discuss the role of reduced muscle contractile activity on the accretion of lipids into IMAT and the potential involvement of the regulation of the cellular metabolic flexibility within muscle on the recruitment of FAPs leading to IMAT accumulation.

Funding: FONDECYT #11190971

S12. HEART FAILURE: MORE THAN A CARDIAC DISEASE

Chairs: Luciana Venturini Rossoni (Brazil), Gerardo García Rivas (Tecnologico de Monterrey, México)

S12-1

Mechanisms underlying the cardiorenal benefits of SGLT2 inhibitors in heart failure

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Sodium/glucose cotransporter 2 (SGLT2) inhibitors, originally designed to improve glycemic control, have unexpectedly demonstrated significant cardiorenal benefits in cardiovascular outcome trials, transcending diabetes status. Various hypotheses have been proposed to unravel the mechanisms underpinning these effects. Our laboratory investigations suggest that alleviating kidney dysfunction may serve as a central mechanism through which SGLT2 inhibitors mitigate heart failure (HF) development and progression. In this presentation, we will provide evidence illustrating that SGLT2 inhibition restores euvolemia in non-diabetic HF rats by preserving glomerular filtration rate (GFR) and renal mass and by inhibiting sodium reabsorption mediated by proximal tubule sodium/hydrogen exchanger 3 (NHE3). Moreover, we will delve into potential mechanisms underlying NHE3 inhibition by the SGLT2 inhibitor empagliflozin (EMPA). Our latest findings shedding light on the renal neurohumoral effects of SGLT2 inhibitors, encompassing the sympathetic nervous system, the renin-angiotensin system, and the shift of inflammatory cells toward the M2 macrophage phenotype, partly mediated through direct effects on macrophages, will also be discussed. Overall, our research has contributed to comprehending the cardiovascular benefits of SGLT2 inhibitors in heart failure.

Financing: This work was funded by grants from the São Paulo State Research Foundation (FAPESP 2021/14534-3) and the National Council for Scientific and Technological Development (304666/2022-0).

S12-2

The excitation-contraction coupling is profoundly altered in ventricular cardiomyocytes of a novel model of Heart Failure with preserved Ejection Fraction

Gerardo Garcia-Rivas[2], Abraham Méndez-Fernández[2], Ángel E. Fernández[2], Leandro A. Díaz-Zegarra[1], Felipe Salazar-Ramírez[2], Alejandro E. Aiello[1]

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Even though heart failure with preserved ejection fraction (HFpEF) represents roughly half of the total cases of heart failure, it is only recently that *bona fide* animal models of HFpEF have been developed. Here, we aimed to characterize the excitation-contraction coupling (ECC) process in a mouse model of HFpEF. HFpEF animals consisted in mice fed with a high-fat diet and exposed to L-NAME for eight weeks. Action potentials and L-type-Ca^{2+} current of cardiomyocytes were recorded via patch clamp. Ca^{2+} transients, Ca^{2+} sparks, and the sarcoplasmic reticulum Ca^{2+} content were measured in myocytes by

confocal microscopy imaging. The T-tubule system was studied in micrographs of di-8-ANNEPS-loaded myocytes. HFpEF myocytes exhibited a dramatic enlargement of the duration of the action potential was 2.2 times higher than CTRL. The amplitude of Ca^{2+} transients was slightly higher, which, however, could not be explained by increases in either L-type-Ca^{2+} current or sarcoplasmic reticulum Ca^{2+} content. Notably, microscopic releases of Ca^{2+}, known as Ca^{2+} sparks, also exhibited a larger amplitude in HFpEF. Of relevance, HFpEF myocytes were characterized by a delayed Ca^{2+} reuptake. This last feature was paralleled by a slower re-lengthening rate. Finally, the release of Ca^{2+} occurred in a less synchronic manner during HFpEF Ca^{2+} transients, despite the T-tubule system regularity and density were both preserved. Ca^{2+} dynamics of HFpEF suggested an impairment in cytosolic Ca^{2+} reuptake, which may underlie diastolic dysfunction. Further research is warranted on the sarcolemmal NCX activity in order to provide a more thorough understanding of the ECC in HFpEF.

Financing: Experimental Research Unit. Institute for Obesity Research. Tec de Monterrey.

S12-3

Vascular dysfunction in heart failure.

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It is well known that heart failure is more than a cardiac disease. Growing evidence demonstrates that endothelial dysfunction is critical in pathogenesis, progression, and organ damage in heart failure. The past 20 years were crucial to include the perivascular adipose tissue (PVAT) as an endocrine tissue able to control smooth muscle cell contractility. This physiological anticontractile effect of PVAT is associated with its ability to release vasoactive substances capable of modifying vascular tonus. Later, this effect was demonstrated to be impaired in the presence of cardiometabolic risk factors in both human and murine models. Interestingly, renin-angiotensin system (RAS) components are expressed in the PVAT arteries of healthy rats, and angiotensin 1-7 is considered one putative vasodilator factor released from PVAT. Previous studies have shown that the activation of the RAS is involved in the pathophysiology of several cardiovascular diseases, such as heart failure. In heart failure animal models and in patients, there is systemic RAS activation as a compensatory mechanism in response to the reduced cardiac output. In addition, such activation was also described in the local RAS, inducing endothelial and PVAT dysfunction. Thus, considering that PVAT has a central role in the control of vascular tone and homeostasis, that RAS components are expressed in PVAT, and that the vascular dysfunction observed in heart failure is associated with RAS activation, this talk will discuss whether RAS overactivation in PVAT is pivotal to the vascular dysfunction in heart failure.

Financing: Foundation for Research Support of the State of Sao Paulo (FAPESP) and National Council for Scientific and Technological Development (CNPq).

S12-4

Brazilian Longitudinal Study of Adult Health (ELSA-Brazil): What are we learning?

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Cardiovascular diseases (CVD) is the most prevalent cause of death in Brazil accounting for around 30% of all deaths, most of them secondary to coronary artery disease (CAD). Age-adjusted rates by heart failure (HF) are also decreasing in the last three decades. However, it remains as the first cause of hospitalization and cost due to CVD. HF is secondary to multiple causes most of them preventable, such as CAD/myocadial infarction, rheumatic cardiac disease, hypertension as well as the Chagas' disease, an usual cause of HF in the some Brazilian regions. The ELSA-Brasil study is a longitudinal study in a professional cohort (civil public employees) that included 15,105 adults of both genders, aged 35-74 years at baseline (2008-2010) aiming at determine proximal and distal factors influencing the incidence of chronic diseases, with focus on CAD, diabetes, cancer,

chronic kidney disease, and neurodegenerative diseases. Clinical and subclinical prevalence of HF was investigated at baseline in participants ≥55 years by echocardiography. The cohort is followed up by annual contacts by telephone, by exams and interviews each 3-4 years and by continuous monitoring of hospital events and deaths. After 10 year follow up (2009-2018), 563 deaths were recorded and the main causes were cancer (39.4%) and CVD (30.7%). From the 1,107 hospitalizations due to CVD in this period, 7.9% were for HF treatment. Further analysis are been planned to determine the association between precocious changes of cardiac structure and function with late outcomes due to HF.

Financing: Ministério da Saúde/Decit e Ministério da Ciência e Tecnologia/CNPq

S13. NEW PHYSIOLOGICAL AND THERAPEUTIC FRONTIERS OF THE INTRA-RENAL RENIN ANGIOTENSIN SYSTEM

Chair: Alexis A González (Pontificia Universidad Católica de Valparaíso, Chile)

S13-1

Non-canonical pathways for renin regulation in the kidney.

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The discovery of renin production within the principal cells of the collecting duct has significantly advanced our comprehension of how intrarenal angiotensin II (Ang II) generation and blood pressure regulation occur. Within the collecting duct, Ang II plays a pivotal role in enhancing renin synthesis and secretion through mechanisms that entail the activation of the Ang II type 1 receptor (AT1R), and the downstream stimulation of the PKCalpha, Ca2+, and cAMP/PKA/CREB pathways. Furthermore, paracrine signaling molecules such as vasopressin (AVP), prostaglandins, bradykinin (BK), and atrial natriuretic peptide (ANP) exert control over renin expression in principal cells. In cases of Ang II-dependent hypertension, where plasma renin activity is suppressed, both renin and the prorenin receptor (RPR) are upregulated within the collecting duct, by fostering the de novo formation of intratubular Ang II. Hence, the interplay between components of the renin-angiotensin system (RAS) and paracrine hormones within the collecting duct creates a dynamic environment for the compartmentalization of the RAS, orchestrating intricate mechanisms that elevate intrarenal Ang II levels, sodium reabsorption, and ultimately, blood pressure.

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S13-2

Antibody-based detection of Angiotensin receptors in the kidney. Challenges and implications in studying regulatory mechanisms.

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The angiotensin II type 1 receptor (AT_1R) mediates most hypertensive actions of angiotensin II however, little is known about the behavior of these receptors and their sub-cellular localization. To understand the molecular mechanisms underlying the regulation of the AT_1 receptor in normal physiology and pathophysiology, methods for sensitive and specific detection of AT_1R protein are required. We will discuss data assessing the specificity of commercial antibodies to accurately detect these receptors across different experimental platforms such as Western blotting with kidney tissue from wild-type mice and genetically modified mice lacking the AT_1R isoforms (AT_1R knockouts). Additionally, we will explore the pattern of immunohistochemistry

staining in kidneys, liver, and adrenal glands of wild-type and AT_1R knockouts.

We will discuss 1) How utilizing non-validated antibodies may lead to erroneous results, 2) How the lack of these tools precludes studying regulatory mechanisms such as receptor internalization and trafficking, and 3) Alternative approaches to overcome the challenges on AT_1R detection.

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S13-3

The Prorenin Receptor (PRR) in Physiology and Its Impact on Hypertension and Diabetes

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The prorenin receptor (PRR), a renin-angiotensin-aldosterone system (RAAS) component, is upregulated during hypertension, obesity-associated hypertension, and diabetes. PRR is expressed as a cell membrane-bound PRR (or full-length PRR), a truncated form, and a soluble PRR (sPRR) processed intracellularly by serine proteases and then secreted to extracellular space. Through a non-catalytic pathway, PRR activates prorenin.

The augmentation of intrarenal angiotensin (Ang) II contributes to developing and maintaining hypertension. In models of experimental hypertension, the stimulation of PRR favors Ang I formation, which is converted into Ang II by angiotensin-converting enzyme. The coordinated actions of collecting duct-derived prorenin and PRR in the collecting duct are critical to regulating blood pressure. Mice with distal nephron-specific deletion of renin display attenuated responses to Ang II-induced hypertension through a decreased epithelial sodium channel (ENaC) activity and expression. Moreover, these mice exhibit alterations in kidney function.

Evidence supports the significant relationships among PRR, obesity, and type 2 diabetes mellitus (T2DM). Blocking PRR reduces weight gain in obese mice, reduces insulinemia, and normalizes hypertriglyceridemia. Plasma sPRR levels are elevated in patients with obesity, hypertension, pre-eclampsia, and T2DM. Sex also influences plasma sPRR levels, where obese women with T2DM display higher PRA and sPRR levels than men. Indeed, plasma sPRR may indicate the status of systemic RAAS activation and the onset of vascular complications during T2DM in a sex-dependent manner. How plasma sPRR contributes to increased systemic RAAS activation and relevance in developing CVD in patients with T2DM, will be discussed.

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S13-4

Metabolic pathways involved in the regulation of the (pro)renin receptor in the renal collecting duct

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The (pro)renin receptor (PRR) is a member of the RAS and a key regulator of distal tubular Na+ reabsorption, impacting on blood pressure. PRR is expressed in intercalated cells of the collecting duct (CD), a segment containing all components of the RAS. PRR binds to prorenin or renin secreted by the neighbor principal CD cell to promote angiotensin (Ang) II formation and Na + reabsorption. During deregulation of the tricarboxylic acid cycle (TCA), some of the

intermediaries, such as alpha-ketoglutarate are accumulated in renal tissues. This phenomenon has been observed in diabetes and during reduced blood supply to the kidney. The receptor for alpha-ketoglutarate. GPR99, also known as oxoglutarate receptor 1 (OXGR1) is expressed in intercalated cells. We have demonstrated that OXGR1 can increase PRR expression in cultured collecting duct cells. Furthermore, the pharmacological blockade of OXGR1 impairs PRR upregulation in mice with Type 1 diabetes. The role of OXGR1 in regulating PRR in the distal nephron segments is unknown. Here, the experimental Goldblatt mice model (two kidneys and one clip 2K1C) was used to reduce renal blood flow and to promote RAS activation, OXGR1 knockout mice and pharmacological blockade of OXGR1 (montelukast) showed attenuated increases in arterial blood pressure. The fact that OXGR1 is abundantly expressed in the collecting duct and that its activation regulates PRR suggests new important pathways regulating intratubular RAS and Na+ reabsorption and impacting on blood pressure.
Financing: FINANCIAMIENTO: FONDECYT 1220525

S14. MINING THE WAVEFORM, NEW APPROACHES TO DELINEATING RESPIRATORY FEATURES AND ANALYZING RESPIRATORY OUTCOMES

Chair: Russell Ray (Baylor College of Medicine, USA)

S14-1

A cognitive framework for applying machine learning to neurophysiological assays

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Modern approaches to neurophysiology research require boundary spanning skillsets not traditionally taught during neurophysiology training. The goal of this presentation is to provide a cognitive framework that will help the attendees learn about different ways to apply the ever-expanding toolbox of informatics tools to their work. The analytical methodologies to be discussed will include principal components analysis, dimensionality reduction, clustering, regression, prediction, and feature selection. This will be illustrated using our recent data from neonatal sepsis models using TLR4 and TLR1/2 ligands. We found that inflammation induced by these pathways result in different gene pathway activations. Specifically, TLR1/2 ligand activation results in a robust immune response without protective metabolic suppression, whereas TLR4 results in a severe metabolic suppression with inflammation. Using machine learning tools, we identify mechanistic candidates that modulate inflammation and metabolism.
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S14-2

Quantification of non-linear variability in cardio-respiratory control: Open-source tools for physiology signal analysis

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Physiological signals provide a continuous index of communication between organ systems. Organ systems communicate with the central nervous system via interoceptors and the central nervous system (CNS), in turn, drives cardiovascular and respiratory rhythms in response to changes in both the internal and external environment. In this presentation, we will provide an overview of free, open-source software (FOSS) specifically developed for the analysis of physiological signals. In addition to work from other groups, we will provide an overview of BASS— the Biological Analysis Software Suite— a suite of tools that we have produced to dissect linear and non-linear components in physiological signals. BASS is an open-source, Python-based set of tools that can be used to analyze plethysmographic, electromyographic, electrocardiographic, and neural data from murine, ovine, and human subjects. BASS encompasses modules for cardiac, respiratory, and imaging data. Special emphasis has been placed on a streamlined, customizable, and reproducible work-flow. BASS has both command line and Jupyter notebook interfaces, allowing for batch analysis of large-scale physiologic datasets as well as exploratory data analysis and visualization. Ultimately, BASS allows

reproducible data analysis, interactive visualization, and rapid creation of high-quality figures for publicastion.

Financing: NIH grants HD092941 (R21) and AT011691 (R01)

Acknowledgments: We greatly appreciate the work of Abby Dobyns, Melisa Custer, Rhaya Johnson, and Samuel Murray in developing BASS.

S14-3

Automated interrogation of waveforms designed for respiratory waveform analysis

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Respiratory dysfunction is a common endpoint in many diseases ranging from anxiety and depression to spinal cord injury to neurodegenerative disorders such as Alzheimer's Disease. Whole body plethysmography (WBP) with concurrent metabolic measurement is a non-invasive, high face-value approach for understanding the critical, yet underappreciated, respiratory component of disease progression and mortality in rodent models. Data derived from WBP experiments are extensive and dense with information. Currently, standard approaches employ manual analysis focused on only a limited number of respiratory and metabolic features sampled from a small portion of the collected waveforms, which leaves the majority of the data unanalyzed and experiments underpowered. Therefore, we sought to develop methods that would allow for robust and in-depth yet facile interrogations of WBP experiments. Our solution, Breathe Easy is an open source, front-to back software solution for the selection and analysis of respiratory data. The software contains three key modules: 1) a user-friendly interface that enables rapid engagement by less experienced investigators while offering extensive menu structures for deeper interrogations by advanced users; 2) a waveform feature extraction module to segment, filter, and quantify pertinent respiratory variables, based in part on investigator-provided values; and 3) an analysis and graphing module that generates publication-worthy graphs and multivariate statistics for operant outcomes. Together, these modules create an innovative, first-of-its-kind software package that advances the field by facilitating automated analyses of respiratory experiments of varying complexity from small experiments using default parameters to comprehensive, deep phenotyping of terabyte-scale multivariate respiratory data.

S14-4

Mining the respiratory waveform of patients with sleep disordered breathing

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Sleep Disordered Breathing is a major health issue in adult and pediatric patient populations. Here we characterize the breathing rhythm in pediatric patients suffering from obstructive sleep apnea, Leigh Syndrome and Rett syndrome. We developed automated algorithms that allowed us to quantitatively characterize the different phases of breathing during sleep and wakefulness. We find that characteristics of inspiratory and postinspiratory activity are differentially modulated during different stages of sleep and wakefulness. These characteristics and their inter individual variability vary dependent on the

breathing disorder. Characterizing the detailed morphology of breaths in a cycle-by-cycle manner provides important insights into the dysregulation of breathing in different patient populations. This is an important step towards a better understanding of the differential pathophysiological mechanisms that underlie these disorders.

Financing: Funded by the Aaron Matthews Guild for Sudden Infant Death Syndrome and the National Institute of Health.

S15. OBESITY AND THE RISK OF CARDIOMETABOLIC DISEASES

Sponsored by Elsevier

Chairs: Gerardo García-Rivas (Tecnológico de Monterrey, México), Marco Rito-Palomares (Tecnológico de Monterrey, Mexico)

S15-1

Previous Cardiovascular Injury is a Prerequisite for Immune Checkpoint Inhibitor-Associated Lethal Myocarditis. A Preclinical Study in Hypertensive Mice.

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Immune Checkpoint Inhibitors (ICIs) activate the immune response against cancer. While ICIs are generally well-tolerated in terms of toxicity, they can lead to various immune-related adverse events (irAEs), especially in combination therapy with αCTLA-4 and αPD-1 inhibitors. Notably, cardiotoxicity and myocarditis have emerged as severe and poorly understood complications of this treatment. **Methods**: To investigate if pre-existing cardiac damage is necessary for the development of ICIs-induced cardiotoxicity, we administered combination therapy with ICIs to a murine model of hypertension (HTN), either as a single dose or over a three-dose regimen spanning 7 and 21 days of ICIs treatment. Our analysis encompassed the following aspects: i) Survival rates, ii) Phenotypic and pathological changes, including cardiac biomarker BNP, iii) Cardiac function assessment through PV loops measurements, iv) Evaluation of cardiac inflammation, and v) Monitoring the progression of myocarditis. **Results**: After the first administration of ICIs combined therapy, the treated HTN group showed increased mortality ($p = 0.0002$) and early hypertrophy and remodeling compared to the untreated HTN group. BNP ($p = 0.01$) and TNF-α (<0.0001) increased also in the treated group, while IL-6 ($p = 0.8336$) remained unchanged. Remarkably, myocarditis only developed in the HTN group treated with ICIs on day 21, characterized by T cell infiltration and increased cardiac antigen antibodies ($p = 0.05$). The control group treated with ICI was unaffected in any evaluated feature. **Conclusion**: Our findings suggest that pre-existing, sustained cardiac damage is a crucial prerequisite for ICI-induced myocarditis.

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S15-2

Fatty liver disease and cardiac dysfunction: perspectives from a preclinical study of heart failure with preserved ejection fraction.

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Ventricular heart disease with non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatosis often manifests clinically as heart failure with preserved ejection fraction (HFpEF). Nowadays, HFpEF with NAFLD prevalence could reach 50%, however, it is neither known which clinical entity appears first nor its intercommunication pathways.

The objective of this study was to determine liver changes in a mouse model of HFpEF and assess the presence of NAFLD and its underlying mechanisms. A HFpEF two-hit model was induced in C57BL/6 mice by administration of L-NAME and high fat diet, and was studied at 0, 8 and 12 weeks. Serum and liver tissue were extracted. Serum was analyzed for HDL, LDL, TG, ALT, AST, albumin, and protein. Liver tissue was assessed for steatosis and fibrosis with H/E and Masson, respectively, triacylglycerols (TG), cholesterol, mitochondrial function, oxidative stress markers, LPO, inflammatory and lipidic markers associated with fatty acids metabolism.

The HFpEF group presented alterations of left ventricle function, impaired diastolic pressure, high stiffness index, and pulmonary congestion. Cardiomyocytes showed hypertrophy and increased expression of natriuretic peptides, indicating pathological remodeling. Liver tissue micronodular steatosis was observed without fibrotic bridges or parenchyma changes. Plasma TG and LDL showed a significant increase; and TG accumulated in the liver but did not induce mitochondrial damage at 8 weeks, whereas it manifested until 12 weeks of HFpEF development. In summary, these time-course dependent alterations, along with intracellular lipid overaccumulation, suggest chronic liver damage.

Financing: The work was supported by Tecnologico de Monterrey through the Experimental Medicine and Advanced Therapies research unit of the Institute for Obesity Research.

S15-3

Changes in [Glucose]e induce cardiac ventricular arrhythmias partially dependent on SGLT1 activity in mice

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Introduction: Obesity is linked to higher risks of cardiac arrhythmias, as well as type 2 diabetes mellitus, where acute changes in [glucose]e (ΔGE) can occur. ΔGE activates Ca2+-Calmodulin kinase II and favors Na+-Ca2+ exchanger-inducing arrhythmogenic events (AE), without considering sodium/glucose cotransporter 1 (SGLT1).

Objectives: Study the role of SGLT1 in the AE induced by ΔGE.

Methods: Ca2+i (CaiT) and developed pressure in myocytes and perfused heart were measured. Preparations were exposed to 5.5mM (low glucose, LG) or 11mM (normal glucose, NG) glucose until stabilization of CaiT, and changed to 11mM or 25mM (high glucose, HG) glucose, respectively. Osmotic controls were performed (choline chloride or sucrose). SGLT1, GLUT1/4, and AMPK were inhibited (Phlorozin 10µM, Dorsomorphin 10µM, and Cytochalasin B 1µM, respectively). Western Blot and electrocardiograms (ECG) were also performed.

Results: AE were observed in 96% of the cells when ΔGE from LG-NG and 76% when ΔGE from NG-HG. In perfused hearts, we observed the same pattern of AE with the ΔGE. AE were absent in the osmotic controls. Phlorozin, significantly prolonged the onset of AE in myocytes. Washout of phlorozin unmasked the ΔGE-induced AE in whole heart. ΔGE increased pGLUT-4, a fact that was counteracted by dorsomorphin. However, inhibition of GLUT1/4 did not increase the latency time. ECG traces showed that i.p. glucose administration trended to increase ventricular extrasystoles in all glucose-treated mice.

Conclusion: The acute ΔGE would induce AE dependent, partially, on SGLT1 activity and independently on osmolarity. These cellular AE could induce ventricular arrhythmias.

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Acknowledgments: Monica Rando

S16. RECENT ADVANCES IN THE PHYSIOLOGY OF THE IMMUNE SYSTEM IN HEALTH AND DISEASE

Chair: Paola Murgas Alcaíno (Chilean Society of Immunology, SOCHIM)

S16-1

The cGAS/STING signaling pathway: Its contribution to the inflammaging process

Paola Murgas Alcaíno[1,2], Camila Sánchez-Pérez[1], Ian Riquelme[1], Daniela Carrillanca[1], Matías Mansilla[1], Martina Jaramillo[1], Fabián Rojas[1], Valentina Frenkel[3], David Ascencios[3], Joaquín Castro[3], Trinidad Pizarro[3]

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Introduction: The cGAS/STING signaling pathway recognizes both endogenous (from mitochondria and the nucleus) and exogenous (from bacteria and viruses) double-stranded DNA in the cytoplasm, releasing proinflammatory cytokines. The cGAS/STING proteins have been implicated in regulating metabolism, generating a proinflammatory state in mice obesity models. However, the underlying mechanism remains unknown together with its role during inflammaging.

Objective: This study aims to investigate the potential involvement of the cGAS/STING pathway in the lipidic metabolism during aging.

Methodology: Different groups of age mice were utilized from Wild-Type (WT), cGAS protein-deficient mice (cGASKO), and STINGKO (bioethics committee code 5/2018, Fondecyt 11190258). The body weight of the experimental groups was assessed, revealing that both cGASKO and STINGKO mice exhibited higher body weight at all ages compared to the WT group. Notably, the cGASKO and STINGKO mice did not consume larger quantities of food than the WT mice at any age, and the KO groups did not exhibit alterations in strength, colon size, or motility. Nevertheless, cGASKO and STINGKO mice of all ages displayed elevated levels of circulating triglycerides and cholesterol, accompanied by increased liver and adipose tissue morphometric size and signs of steatosis. These findings suggest that in the absence of cGAS and STING proteins, lipids accumulate in metabolic tissues during aging. Statistical analyses were conducted using Two-Way ANOVA with n=3-9 mice per experimental condition, and the results are presented as mean ± SEM.

Conclusions: Our findings demonstrate the involvement of the cGAS/STING signaling pathway in lipid metabolism during the aging process.

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Acknowledgments: Consorcio Ci2030, Facultad de Ciencias, Universidad Austral de Chile (UACh)Fondecyt 11190258

S16-2

Beyond the Central Dogma: Exploring the world of LncRNAs and their impact on immunity against bacterial pathogens

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Traditionally, the concept of the Central Dogma of Molecular Biology has illustrated the flow of information from genes to proteins. However, a significant portion of the genome is transcribed into functional RNAs with low or no potential for translation (non-coding RNAs). Long non-coding RNAs (lncRNAs) represent the largest group of non-coding RNAs and play a role in regulating most biological processes, including the immune response. In this talk, we will describe the mechanisms and the role that lncRNAs play in the activation and suppression of genes involved in immunity against bacterial pathogens, influencing cytokine production, immune cell activation, and inflammation. Finally, we will discuss the importance of looking beyond the "Central Dogma" and how the study of lncRNAs can open new perspectives in the research and treatment of infectious diseases.

Financing: Fondecyt 1230018

S16-3

Equine Asthma: a model of neutrophilic phenotype asthma in humans.

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Equine asthma is a human asthma-like condition that develops in mature horses following stabling and exposure to dusty hay and straw. The term "equine asthma" encompasses the previous names of recurrent airway obstruction (RAO) and inflammatory airway disease (IAD), which are now considered moderate-to-severe and mild forms, respectively, of a single disease; moreover several authors suggest that equine asthma is the study model of neutrophilic asthma in humans. Aspergillus fumigatus, an opportunistic fungus, is commonly found in horses´ environments and is considered one of the inciting agents in equine asthma. Asthma-affected horses respond to this exposure by developing an increase in airflow resistance due to neutrophilic inflammation, mucus accumulation, and airway hyper-responsiveness, with a decrease in pulmonary function1. In general, airway inflammation in asthma horses involves the activation of pathogenic specific inflammatory cells, modulation of gene transcription factors and release of inflammatory mediators. Type I hypersensitivity, which is IgE-mediated, and type III hypersensitivity reactions have been suggested to play a role in airway inflammation. T cells also play an important role in the modulation of the immune response in equine asthma pathogenesis. Some findings suggest that pulmonary helper T lymphocytes may be implicated in equine asthma through the secretion of Th1-type or Th2-type cytokines. IL-17 is produced by the subset of T cells termed Th17 cells; this subset of T cells seems to have a role in immediate influx of neutrophils into the airways of asthma-affected horses.

Financing: FONDECYT 1230101

S17. PEPTIDE MODULATION IN SYSTEMS PHYSIOLOGY

A symposium sponsored by the International Regulatory Peptide Society, affiliated to IUPS

Chairs: Limei Zhang (Universidad Nacional Autónoma de México, Mexico), Valery Grinevich (Heidelberg University, Germany)

S17-1

Oxytocin protects nigrostriatal dopamine system in Parkinson's disease model

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The most pronounced neuropathological feature of Parkinson's disease (PD) is the loss of dopamine (DA) neurons in substantia nigra (SN), which depletes striatal DA. Hypothalamic oxytocin is found to be reduced in PD patient and closely interact with DA system, but the role of oxytocin in PD remains unclear. Here, we observed the disturbances of endogenous oxytocin level and SN oxytocin receptor expression in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model, correlated with striatal tyrosine hydroxylase (TH) expression reduction. Killing/silencing hypothalamic oxytocin neurons aggravates the vulnerability of nigrostriatal DA signal to MPTP, whereas elevating oxytocin level promotes the resistance. Knocking down SN oxytocin receptors induces the time-dependent reductions of DA neurons and striatal DA level by increasing neuronal excitotoxicity. Our results further uncover that oxytocin dampens the excitatory synaptic inputs onto DA neurons via activating oxytocin receptor-expressed SN GABA neurons, which target GABA(B) receptors expressed in glutamatergic axons. Thus, oxytocin acts as a key endogenous factor in protecting the nigrostriatal DA system.

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S17-2

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Neurocircuitry for Endocrine and Behavioral Stress Responses

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The neuropeptide PACAP is a master regulator of central and peripheral stress responses. PACAP/PAC1 receptor signaling has been implicated in the pathophysiology of

neuropsychiatric disorders related to stress. PACAP neuronal circuits within cortical and limbic areas, hypothalamus and brain stem, including the external lateral parabrachial nucleus (elPBn), have been implicated in the regulation of stress coping and defensive behaviors. We used anatomically specific viral injection in PACAP-Cre and PACAP floxed mice to identify the PACAP-containing circuits that mediate behavioral responses, and hypothalamo-pituitary adrenal (HPA) axis activation, following acute restraint stress. Our studies identify PACAPergic circuits that separately control endocrine and behavioral stress responses. A PACAPergic projection from the elPBn to the PKCδ neurons in extended amygdala contributes to acute stress-induced hypophagia. Strikingly, HPA axis activation by acute restraint stress is completely independent of PACAP depletion in this parabrachial-to-amygdalar projection. A second PACAPergic pathway from frontal cortex directly to hypothalamus regulates the endocrine stress response by regulating expression of corticotrophin releasing hormone (CRH) which controls ACTH secretion from the pituitary, and subsequent CORT secretion from the adrenal cortex. Depletion of PACAP from this pathway blunts activation of CRH neurons and CRH biosysthesis in paraventricular nucleus of the hypothalamus (PVN) without affecting the behavior (hypophagia) after acute restraint stress. Defining two separate limbs of the acute stress response provides broader insight into the specific brain circuitry engaged by the psychogenic stress response, and a potential alternative view to the hypercortisolemia hypothesis for explaining the stress-induced behavioral responses potentially underlying human melancholic depression.

Financing: National Institute of Mental Health Intramural Research Program, Project MH002386

S17-3

Novel neuropeptide roles in synaptic structure and synaptic pruning in hippocampus: effects of vasopressin on PSD proteins and microglial activity

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Neuropeptides are abundantly expressed in the brain, especially in limbic regions in which they are often expressed in GABAergic and glutamatergic subpopulations which serve as useful markers for studies for those subpopulations. It is well established that neuropeptides act as extra-synaptic chemical messengers for brain function. However, for most neuropeptides, it is not known how or when they are released from axon terminals in relation to the canonical neurotransmitters, such as GABA and glutamate, with which they may be co-released.

The focus of this talk will be on the modulatory role of the neuropeptide arginine vasopressin (AVP) through cell-type specific innervation, modification of the postsynaptic density proteins PSD95/GLUA1 and synaptic pruning onto the spines of axon initial segments in hippocampal formation, as well as its potential role on behavioral output, especially during stress coping.

A brief literature review will be followed by presentation of observations from our research groups in recent years. The intent is not to minimize neuropeptide roles in extra- synaptic function, nor the involvement of the amygdala and other brain regions in stress coping and fear memory, but to provide examples for future hypothesis generation and studies toward the comprehensive understanding of the roles of neuropeptides, like vasopressin, in systems neurophysiology.

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S17-4

Using a novel transgenic AVP-Cre rat to dissect arginine vasopressin circuits in the brain and their behavioral roles

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Arginine vasopressin (AVP) is a neuropeptide produced by magnocellular AVP neurons in the hypothalamus. It has important hormonal functions of homeostatic regulation, but importantly it also has neurological functions on physiology and behavior, which are conveyed via axonal projections to many brain centers. To study these functions in detail, it is important to be able to target specific AVP subpopulations to study their connectivity and functions. Here we present a novel transgenic AVP-IRES2-Cre knock-in Sprague-Dawley rat, generated by CRISPR/Cas9-mediated targeted insertion of IRES2-Cre into the 3' untranslated region of the AVP gene. This rat allows us to neuroanatomically dissect and neurophysiologically manipulate specific AVP circuits in the brain using viral vectors. We will show how we are currently applying these new abilities to systematically map the connectivity of AVP neurons throughout the brain, to perform ex vivo patch-clamp electrophysiology of virally tagged AVP neurons, and to perform in vivo calcium imaging of AVP neurons by fiber photometry. Lastly, we will show preliminary results of how we employ our new rat to dissect the AVP-to-central amygdala circuit and study its behavioral functions using optogenetics and behavioral paradigms. Our preliminary results demonstrate the power of our novel AVP-Cre rat model as a tool to take AVP research to a new level, to explore in detail the largely unknown complex neuroanatomy and neural functions of AVP-ergic circuits throughout the brain.

S18. CONNECTING STUDENTS WITH THE COMMUNITY TO ENHANCE LEARNING

Chairs: Patricia A. Halpin (University of New Hampshire, USA), Victoria Velarde (Universidad de Valparaíso, Chile)

S18-1

Using a Role Play Activity with Life Science and American Sign Language (ASL) Interpreting Students to Provide Undergraduates Experience in The Healthcare Setting

Patricia Halpin[1]

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Providing students with real-life professional experiences increases their confidence and ability. Life science undergraduate students in an endocrinology course joined the ASL Interpreting students to participate in a robust learning activity. The life science student played the role of a healthcare provider while one ASL Interpreting student played a deaf patient and the other played the ASL interpreter. Classrooms were set up as seven unique clinics and the activity was performed over two evenings. The clinics were pediatrics, emergency department admissions, general practitioner/primary care clinic, diabetes clinic, cardiac clinic, in-patient discharge, and asthma clinic. This authentic learning experience increased the confidence of all the students as well as increased their preparation for their future careers.

S18-2

Reflective Diaries as a Tool for the Development of Metacognition in Students from a Physiology Course

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In the implementation of service learning (A+S), the continuous process of reflection is essential for the critical analysis of the service activity that has been performed. For this reason, we implemented an A+S activity with a reflective diary in the course "Laboratory of physiology" at the Pontificia Universidad Católica de Chile. The construction of a reflective diary as a method for the systematization of the reflective process was also useful as an evaluation tool. The service activity involved the design of a physiology laboratory activity, in which our university students had to transfer biological content and scientific thinking skills addressed in the course, to students from vulnerable high schools in Santiago, Chile.

The possibility of reflecting on the work carried out with high school students allowed the university students to be aware of their own perception about what they have learned about themselves, and the contribution made to the school community.

The students recognized that they had enhanced their communication, teamwork, and social commitment skills and that it is necessary for a biologist to develop the soft skills worked in the course. Some of the observations made by our students, that account for the process of metacognition performed by them in their diaries, are presented as examples.

The students valued positively the contribution of the service-learning activity to their personal development, as they were challenged to make scientific dissemination of physiological concepts to different social realities.

Financing: Funding: Facultad de Ciencias Biológicas, PUC

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S18-3

IUPS efforts stimulate broader community engagement

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The Education Committee of the International Union of Physiological Sciences (IUPS) engages teachers and learners to create vibrant community. For the past 50 years, the Teaching Workshop tied to the main IUPS Congress has attracted teachers to an intensive faculty development experience. The model of appending a focused Teaching Workshop to regional meetings has been extended, and now workshops are tied to the meetings of PANAM, FAOPS, AAPS and SAAP. A collaboration with the Education Committee and the IUPS Board of the General Assembly produced a 4-workshop series on “Physiology Education Techniques” across India, supporting the shift to competency-based education and learner-focused instruction in that country. The IUPS also helps promote the Inter Medical School Physiology Quiz (IMSPQ) developed in Malaysia by Hwee-Ming Cheng in 2003. There are now 13 national competitions, and as COVID restrictions were lifted, the major in-person international competition resumed in 2023. In summary, the platform provided by the IUPS allows dedicated physiology educators to network and refine their individual skills, engaging the community of educators and students.

S19. RECENT ADVANCES AND FUTURE AVENUES IN UNDERSTANDING OBESITY AS A PREMATURE AGING PHENOTYPE

Chairs: María Paulina Correa (INTA, Universidad de Chile, Chile), Christian González-Billault (Universidad de Chile, Chile)

NOTE: S19 Cancelled by the Chairs (Nov 14, 2023)

S20. NEW PATHOLOGICAL MECHANISMS OF CARDIOVASCULAR DISEASES

Chairs: Sergio Lavandero (Universidad de Chile, Chile), Mario Chiong (Universidad de Chile, Chile)

S20-1

Cardioprotection by endothelial small extracellular vesicles

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Small extracellular vesicles (sEVs) are nanosized structures than can carry signals, such as proteins, lipids, RNA and DNA from one cell to another. In recent years, the study of sEVs has gained widespread attention from the scientific community, for their potential as biomarkers and therapeutic agents. The early observation that plasma sEVs can confer cardioprotection against ischemia/reperfusion (I/R) injury paved the way towards the assessment of endothelial sEVs, to identify the source of these cardioprotective vesicles. Endothelial cells control vascular tone, permeability, inflammation, angiogenesis, among other physiological functions. Currently, it has been shown that endothelial cells also release functional sEVs that may have relevant roles in cardiovascular diseases. Interestingly, the physiological state of the cells can alter the production and function of endothelial sEVs. Our group has shown that these sEVs can reduce cardiomyocyte cell death in both *in vitro* and *ex vivo* I/R experimental models. However, our findings also show that a pro-inflammatory environment in endothelial cells impairs the cardioprotective effect of sEVs. In addition, pharmacological treatment of endothelial cells with a cardioprotective agent provides further protection elicited by these vesicles. Our research has also shown that endothelial sEVs may attenuate the pathophysiological phenotype of vascular smooth muscle cells induced by a pro-inflammatory stimulus, suggesting a protective role beyond the protection against acute myocardial infarction.

Financing: Fondecyt 1231576, FONDAP 15130011 (Jaime Riquelme Meléndez).

S20-2

Role of the mitochondrial protease ClpXP in the vasculature

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We have established that the nuclear-encoded mitochondrial protein Poldip2 promotes VSMC differentiation both in vivo and in vitro. Our recent investigations reveal that Poldip2 modulates cellular metabolism by controlling a novel pathway related to the lipoylation of mitochondrial proteins. This process is governed by a mechanism that influences the activity of the caseinolytic peptidase (Clp)-protease complex (ClpXP). Based on these findings, we investigated the role of the mitochondrial protease ClpXP in the regulation of the VSMC phenotype.

Using both gain-of-function and loss-of-function approaches, we demonstrated that ClpXP activity induces a differentiated and anti-inflammatory phenotype in VSMCs. Mechanistic insights indicate that this ClpXP-mediated VSMC phenotype transformation results from the modulation of the cellular NAD+/NADH balance and the subsequent activation of SIRT1. Importantly, pharmacological activation of ClpXP with the small molecule TIC10, which is currently in clinical trials for cancer therapy, replicates this highly differentiated and anti-inflammatory VSMC phenotype both in vitro and in vivo. Such activation significantly diminishes aneurysm formation and preserves vascular architecture in an elastase-induced mouse model of aortic aneurysms.

In summary, our research suggests that ClpXP serves as a novel target in modulating the VSMC phenotype both in vitro and in vivo. Additionally, pharmacological activation of ClpXP reduces aneurysm formation in mice. Given these findings, we propose that TIC10 might be considered for repurposing in the treatment of human aneurysms.

Financing: This study was supported by National Institute of Health through Awards HL095070 and HL113167.

S20-3

Primary cilia in cardiac fibrosis

Elisa Villalobos[1,2], Alfredo Criollo[1,2], **Sergio Lavandero**[1,2]

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Primary cilia are microtubule-based, hair-like structures present on the surface of most mammalian cells, including cardiac fibroblasts. Whereas ciliated cells have been described in developing heart, a role for primary cilia in the adult heart has not been reported. This, coupled with mutations in genes coding for multiple ciliary proteins, underlie polycystic kidney disease, a disorder with numerous cardiovascular manifestations. We aimed to identify cells in the adult heart harboring a primary cilium and to determine whether primary cilia play a role in disease-related remodeling. We identified primary cilia in mice, rats, and human hearts, specifically and exclusively in cardiac fibroblasts. Ciliated fibroblasts are enriched in areas of myocardial injury. Transforming growth factor β-1 signaling and SMAD3 activation were impaired in fibroblasts depleted of the primary cilium. Extracellular matrix protein levels and contractile function were also damaged. In vivo, depletion of PC1 in activated fibroblasts after myocardial infarction impaired the remodeling response. We concluded that fibroblasts in the neonatal and adult heart harbor a primary cilium. This organelle and its requisite signaling protein, polycystin-1, PC1, are required for critical elements of fibrogenesis, including transforming growth factor β-1-SMAD3 activation, production of extracellular matrix proteins, and cell contractility. Together, these findings point to a pivotal role of this organelle and PC1, in disease-related pathological cardiac remodeling.

Financing: FONDAP 15130011

S20-4

New insight of the renin angiotensin system on vascular remodeling

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The renin-angiotensin system (RAS) is involved in the regulation of vascular remodeling induced by hypertension, vascular injury, and inflammation. Angiotensin II (Ang II) is the most important peptide of the classical RAS pathway. Ang II exerts its biological action by binding to AT1 (AT1R) and AT2 (AT2R) receptors. The discovery of the homologous angiotensin I converting enzyme (ACE2) unmasked an intricate system of RAS regulation. In the counter-regulatory RAS pathway, Ang-(1-7) binds and activates the Mas receptor (MasR), while Ang-(1-9) binds and activates AT2R. We demonstrated that Ang II induces vascular smooth muscle cell (VSMC) hypertrophy through an autophagy-dependent mechanism. However, Ang-(1-9), through an AT2R/Akt/FoxO1-dependent mechanism, inhibits platelet-derived growth factor-BB (PDGF-BB)-dependent VSMC dedifferentiation and Ang II-dependent VSMC hypertrophy. Because of these effects, the counter-regulatory RAS emerges as a suitable therapeutic alternative to treat vascular diseases.

Financing: This work was supported by the Agencia Nacional de Investigación y Desarrollo (ANID) Chile (FONDECYT 1220392 and FONDAP 15130011).

S21. NEW INSIGHTS INTO THE STUDY OF ADAPTIVE AND MALADAPTIVE MYOCARDIAL GROWTH

ISHR LAT symposium

Chairs: Martín Vila Petroff (CONICET-UNLP, Argentina), Irene L. Ennis (CONICET-UNLP, Argentina)

S21-1

Regulation of mitochondrial function and morphology during cardiomyocyte adaptative growth

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Mitochondria play a critical role in the heart, as they generate the energy (ATP) required for the heart's continuous contraction and blood pumping. The function and morphology of mitochondria are of utmost importance for maintaining cardiac performance, especially during increased demand. As the heart experiences increased workloads, the need for ATP rises. To meet this demand, mitochondria adjust their function by enhancing ATP production. Various regulatory mechanisms optimize the production of ATP while minimizing reactive oxygen species (ROS) generation. Mitochondrial biogenesis, which involves the creation of new mitochondria, also contributes to increased energy production. Morphologically, the shape and arrangement of mitochondria in cardiac cells adapt to support their function. Mitochondria often form elongated networks that align with sarcomeres, the contractile units of cardiomyocytes. This arrangement optimizes ATP supply to the contractile machinery. Changes in mitochondrial morphology are regulated by fusion and fission processes, enabling mitochondria to distribute and maintain their health. Fusion facilitates the exchange of genetic and protein content, while fission segregates damaged mitochondria for removal. During ischemia, mitochondria may undergo morphological changes that disrupt their normal function, leading to energy deficits and contributing to heart dysfunction. Understanding the intricate balance of mitochondrial function and morphology in the heart is crucial for maintaining cardiac health and adapting to various physiological and pathological stressors. In this talk, I will discuss our main findings in mitochondrial function and morphology during cardiac hypertrophy and heart failure development.

Financing: FONDECYT 1200490 (SL), FONDAP 15130011 (SL)

S21-2

Role of the alkalinizing transporters in the development of pathological cardiac hypertrophy.

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Cardiac cells depend on specific sarcolemmal ion transporters to assure the correct intracellular pH regulation. Two alkalinizing mechanisms coexist in cardiac myocytes to maintain the intracellular pH: sodium/bicarbonate cotransporter (NBC; electroneutral isoform NBCn1, $1Na^+:1HCO_3^-$ and electrogenic isoform NBCe1, $1Na^+:2HCO_3^-$) and sodium/proton exchanger (NHE1). In addition to regulating intracellular pH, these transporters are a source of sodium influx, which chronically enhances intracellular Ca^{2+}, inducing maladaptive cardiac hypertrophy (CH). Due to its stoichiometry, NBCn1 contributes one Na^+ per HCO_3^- in each transport cycle, while NBCe1 uses only half of the Na^+ per HCO_3^-. Thus, we hypothesized that overactivation of NBCn1 or NHE1, but not NBCe1, could be deleterious. Consistently, we recently reported that the downregulation of the NBCe1 induced CH. To specifically reduce NBCe1, we used shRNA cloned into a cardiotropic adeno-associated vector (AAV9-shNBCe1). Strikingly, the downregulation of NBCe1 causes significant hypertrophic heart growth, lengthening of the action potential in isolated myocytes, increase in the duration of the QT interval and increase in the frequency of Ca^{2+} waves without any significant changes in Ca^{2+} transients. An increased compensatory expression of NBCn1 and NHE1 was also found. We conclude that the reduction of NBCe1 is sufficient to induce CH and modify electrical features of the rat heart. Increasing knowledge about the role of each of the NBC isoforms could be of great importance as possible therapeutic targets for treating CH.

Financing: CONICET and FONCyT, Argentina.

S21-3

Mitochondrial dysfunction in cardiac hypertrophy and failure: chicken or egg?

Judith Bernal-Ramirez[1,2], Hugo Alves-Figueiredo[1,2,3], Christian Silva-Platas[1,2], Yuriana Oropeza-Almazán[1], Martin Ramos-González[1], Eduardo Vázquez-Garza[1,2], Felipe Salazar-Ramírez[1], Abraham Méndez-Fernández[1], Noemí Garcia[1,2,3], Omar Lozano[1,2,3], Guillermo Torre-Amione[1,3,4], Gerardo Garcia-Rivas[1,2,3]

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Prominent alterations in heart failure (HF) are impairment of intracellular Ca^{2+} homeostasis and bioenergetics. In normal conditions, Ca^{2+} management harmonizes the cellular contractility to the energy demand in the myocardial cell, being the mitochondrial calcium (mCa^{2+}) dynamics a relevant process to maintain a connection between the heart's energy provision and the demand for contractile activity. The transport of Ca^{2+} ions within mitochondria is facilitated by the mitochondrial Ca^{2+} uniporter (MCU). This protein complex, located at the inner mitochondria membrane, is composed of a central pore and regulatory subunits responsible for overseeing the control of Ca^{2+} passage and tolerance. While its physiological relevance has been confirmed by inhibiting pharmacologically its activity and decreasing its expression, the role of MCU in energy dysfunction and pathological remodeling by HF development is not well understood. Investigating the function of MCU in the context of HF's underlying mechanisms can provide valuable insights for treating HF and identifying new potential therapeutic targets.

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S21-4

Apelin signalling pathway as a mediator of cardioprotection in the hypertrophied myocardium.

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In the scenery of cardiovascular diseases (hypertension, aortic stenosis, myocardial infarction), a maladaptive myocardial growth, known as pathological hypertrophy, takes place that eventually progresses to heart failure. In contrast, exercise training induces an adaptive growth of the myocardium, called physiological cardiac hypertrophy. This latter involves the production and release of several growth factors and other humoral mediators, among which deserve special attention the insulin-like growth factor 1 (IGF-1) and apelin. Both humoral factors activate the signaling pathway through their receptor: IGF1R and APJ, respectively. We have previously demonstrated that a swimming routine induces the conversion of pathological into physiological cardiac hypertrophy in spontaneously hypertensive rats ISHR) (Garciarena CD et al. Hypertension, 2009 DOI: 10.1161/HYPERTENSIONAHA.108.126805). Moreover, exposure of cardiomyocytes to IGF-1, emulating the swimming routine, produced positive inotropic and antioxidant effects and inhibition of NHE-1 hyperactivity (Yeves AM, et al. Acta Physiologica, 2018 DOI: 10.1111/apha.13092). Since both apelin and IGF-1 show similar cardioprotective effects, namely improvement of cardiomyocyte contractility and antioxidant capacity, we hypothesized that they might share the same cellular pathway, being this the activation of the apelinergic pathway. In line with this, we propose that exposure to IGF-1 could stimulate the translocation of APJ and apelin, stored in intracellular vesicles, to the plasma membrane.

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S22. NOCICEPTION AND PAIN: FROM MECHANISMS TO THERAPEUTIC APPROACHES

Chairs: Trinidad Mariqueo (Universidad de Talca, Chile), Carolina A Oliva (Universidad Autónoma de Chile)

S22-1

Molecular and cellular elements of thermal sensitivity in thermoTRP channels and their modulation as a therapeutic target for pain relief

Karen Castillo[1,2], Karina Carvajal-Zamorano[2,7], Cesar Amaya-Rodríguez[2,3,8], Mauricio Bedoya[1,5], Juan Ferrada[2,9], Carlos Ancatén-González[2,7], Nicolás Ardiles[2], Wladimir Plaza[2,7], Andrés E Chávez[2,6], Pablo Moya[2,4], Ramón Latorre[2,6]

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Temperature sensitivity is enabled by the expression of a subset of transient receptor potential (TRP) ion channels that are activated at different thresholds by cold or hot temperatures. Temperature sensitivity is closely associated with pain sensation in several pathological conditions, and thus thermoTRP channels have been a focus of biomedical research as a target for pain relief. TRP melastatin receptor type 8 (TRPM8) and TRP vanilloid type 1 (TRPV1) channels have been identified as cold and heat receptors in vitro and in vivo, respectively. Both are associated with abnormal cold and heat sensitivity in inflammatory and neuropathic pain conditions and are aberrantly expressed in several cancers. Targeting both receptors has analgesic effects that may counteract pain. We have determined that the coiled-coil domain of TRPM8 contains the molecular elements for temperature sensing in the TRPM8 channel. The molecular determinants for temperature sensing in TRPV1 are more controversial. Analogs of capsaicin, the pungent compound found in chili peppers, have been extensively developed as analgesics; however, they are not effiective in neuropathic pain conditions where the channels are sensitized. In this scenario, inhibitors may be better for pain management. We found that a class of bisphosphonates(BPs) inhibits the TRPV1 channel in vitro and in vivo. Understanding the mechanism of inhibition of TRPV1 by BPs will allow us to uderstand the complex allosteric regulation of the channel, and it will open a new avenue for the design of analgesic compounds for therapeutic intervention in pain and other thermoTRP-related pathologies.

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S22-2

The role of inhibitory currents in sex-dependent pain perception processing in the central amygdala

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In the brain, glial cells release interleukin 1β (IL-1β), which has essential roles in neuroinflammation. The central nucleus of the amygdala (CeA) participates in receiving and processing pain information. Interestingly, this nucleus is enriched in the regulatory auxiliary glycine receptor (GlyR) β subunit (βGlyR) which is modulated by IL-1β. We hypothesized whether the modulation of glycine-mediated inhibitory activity via IL-1β differentially modulates the perception of pain of females and males. We performed in silico docking experiments and identified regions in the α1βGlyR 3D structure (the most predominant GlyR conformation in CNS) where IL-1β can bind. Transfected HEK cells show that the application of IL-1β slows down the recovery of glycine-activated currents in the α1βWT. A similar effect was observed in CeA slices, where the decay constant significantly increases in response to IL-1β. The effect of IL-1β was abolished in channels containing the mutation Y240A in βGlyR, supporting the idea that IL-1β interacts with the GlyR. To understand the physiological relevance of IL-1β modulation, we recorded GlyR currents from CeA slices of a neuropathic pain model. Strikingly, in these animals the GlyR current amplitudes showed a bimodal distribution, unlike the normal distribution observed in control animals, whereas IL-1β significantly reduces the higher amplitude components, suggesting disaggregation of receptor clustering. These findings suggested that the modulation of glycinergic currents induced by IL-1β may be relevant for pain processing in the CeA, modulating the integrated summation and output to and from CeA and, thus, the emotional perception of pain in females and males.

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S22-3

Animal models to measure itching, acute, chronic and neuropathic pain and their use for novel drug development.

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Unlike rodent models of psychiatric and neurological disorders, animal models of pain are arguably more translatable to humans and have been useful for the development of potential novel pharmacological targets and treatments. Here we will show different examples of the use of rodent models of itching, acute and chronic, as well as neuropathic pain for novel drug discovery targeting voltage dependent sodium channels (NaV) and the transient receptor potential vanilloid 1 (TRPV1).

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S22-4

The role of inhibitory glycinergic synapsis of central amygdala in the neuroimmune modulation of chronic pain

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Glycine receptors (GlyR) are inhibitory receptors expressed in the spinal cord and supra spinal areas related to the control of nociceptive signals. Proinflammatory molecules such as Interleukin-1β (IL-1β) have a critical role in the progression of chronic pain. In the present work, we characterized the neuroimmune modulation of GlyRs by IL-1β using bioinformatics, molecular biology, electrophysiological and behavioral approaches in animal models of chronic pain. We overexpressed the subunits of GlyRs in HEK 296 cells and performed patch clamp electrophysiological recordings evoking glycinergic currents in the presence of IL-1β (10 ng/mL). We found that IL-1β reduced the amplitude of evoked glycinergic currents. Tyrosine 240 located at the C loop motif of the auxiliary

beta GlyR subunit played a critical role for this modulation as its replacement for alanine abolished the effects of IL-1β. We then analyzed the effects of IL-1β by electrophysiological patch clamp recordings in central amygdala (CeA) slices. CeA plays a role in spontaneous inflammatory pain responses. Our results showed that IL-1β reduced the amplitude of glycinergic sIPSCs in CeA slices. When we compared these results with those obtained in a neuropathic pain rat model, we found that the chronic sciatic constriction injury was associated with a bimodal amplitude distribution of glycinergic currents with small and large amplitudes. Our results suggest that GlyRs interact with IL-1β and the neuroimmune modulation of GlyRs can regulate the central circuits involved in the nociceptive perception.

Financing: FONDECYT 11220157

S23. PATHOPHYSIOLOGY SIGNALLING MECHANISMS IN DISEASES

Chair: Daniel Peluffo (Universidad de la República, Uruguay)

S23-1

Endothelial Connexin 43 hemichannels: a key relaxation vascular component in female breeder mice

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Blood pressure regulation primarily occurs in arterioles, with the endothelium playing a crucial role by producing vasodilators and vasoconstrictors. Key vasodilators in arterioles are nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), particularly influential in small arteries, affecting blood pressure and blood flow distribution. The exact biochemistry of EDHF remains a subject of debate. However, it involves the activation of Ca^{2+}-activated K^+ channels (K_{Ca}), including small (SK_{Ca}) and intermediate (IK_{Ca}) conductance channels, resulting in endothelium-derived hyperpolarization (EDH) and vasodilation in resistance arteries. Both NO and EDH rely on increased endothelial Ca^{2+} levels. NO modulates endothelial function through guanylyl cyclase 1 (GC1) or protein S-nitrosylation, an underexplored mechanism for regulating vasomotor tone. Connexin hemichannels are found in various cell types' non-junctional plasma membranes, including endothelial cells in peripheral arterioles expressing specific connexin proteins like Cx37, Cx40, and Cx43. Research based on primary cultured endothelial cells and isolated mesenteric arteries underscores the significant role of S-nitrosylated Cx43 hemichannels in endothelium-derived hyperpolarization (EDH) signaling, particularly among female breeders. This study aims to unravel the mechanisms involving endothelial Cx43 hemichannels, especially their role in increasing Ca^{2+} levels that promote endothelial hyperpolarization in female breeders. Additionally, it is hypothesized that Cx43 hemichannel currents activate Ca^{2+}-activated K^+ channels (SK_{Ca} and IK_{Ca}), contributing to endothelial hyperpolarization.

Financing: Career Development Award (AHA number 932684)Research Supplement to Promote Diversity in Science from the AHA (AHA number 23DIVSUP1054931)

S23-2

Role of NOX2 in dystrophic cardiomyopathy

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Introduction: Duchenne muscular dystrophy (DMD) is a fatal, progressive genetic disease that causes cardiomyopathy, characterized by oxidative stress mainly from NADPH oxidase 2 (NOX2). Oxidative stress interferes with normal connexin 43 (Cx43) location to intercalated discs, where it forms gap junctions. In the plasmalemma, Cx43 forms hemichannels, a potential pathway for arrhythmias and tissue damage.

Objectives: To test the hypothesis that nitroso-redox imbalance due to NOX2 activity causes

lateralization of Cx43, inducing hemichannels activity, increasing arrhythmogenicity and myocyte apoptosis in DMD.

Methods: We used 10 months old *mdx* mice, a model of DMD (Committee of Bioethics, Universidad de Talca N°2015-03-A). Contractility was evaluated in isolated hearts and isolated myocytes using NOX2 inhibitors apocynin, vasp2870 and the novel compounds C6 and C14, synthesized by our group. Data was analyzed by ANOVA.

Results: Hearts from mdx mice presented increased NOX2 activity and oxidative stress, reduced contractility and higher number of arrhythmic episodes ($p<0.05$), which was associated with an increased number of apoptotic cells and increased fibrosis ($p<0.05$). These conditions were reversed when *mdx* mice were treated 1 month with apocynin. While total cardiac Cx43 content was unchanged, dystrophic hearts showed more Cx43 at lateral membranes. Hemichannels opening was substantially higher in *mdx* hearts and was normalized when mice were treated with apocynin or acutely using carbenoxolone. In addition, *mdx* hearts exhibited increased S-nitrosylation of Cx43 that was reversed by apocynin.

Conclusions: In DMD, increased NOX2 activity deregulates Cx43 distribution, causing hemichannels formation, apoptosis and cardiac dysfunction. These effects are revered by pharmacological NOX2 inhibition.

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S23-3

Modulation of L-arginine transport by nitric oxide: pathophysiological implications

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Plasma membrane cationic amino acid transporters (CATs) mediate L-arginine (L-Arg) transport in cardiac myocytes and other cell types that lack endogenous synthesis of this key amino acid. In turn, L-Arg is the substrate for intracellular nitric oxide (NO) production by the enzyme Nitric Oxide Synthase (NOS). NO shows opposite effects on cardiac muscle contraction and relaxation, depending on the local environment where this potent vasodilator and second messenger is produced. Furthermore, too little or too much NO is detrimental for cell function, as in Duchenne Muscular Dystrophy cardiomyopathy and cardiomyocyte apoptosis, respectively. A decade ago, we reported a physiologically-relevant negative feedback mechanism in which NO modulates its own biosynthesis by inhibiting L-Arg import via CATs. High- and low-affinity L-Arg transporters are inhibited by NO with 3-fold differences in Ki values, although both in the nanomolar range. In this presentation, we will describe novel effects produced by NOS byproducts on cationic amino acid transport and its implications for cardiac myocyte pathophysiology.

S23-4

Adenosine/L-arginine-NO signalling in human placenta endothelium from gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) causes endothelial dysfunction at the macrocirculation in the human placenta. Since the blood level of adenosine in the umbilical vein, but not in arteries, is higher in GDM compared with normal pregnancies, a role for this endogenous nucleoside in GDM-associated endothelial dysfunction is proposed. Adenosine uptake is mediated via the human equilibrative nucleoside transporters 1 and

2 in human umbilical vein endothelial cells (HUVECs). The expression (SLC29A1 gene) and activity hENT1 are differentially modulated by insulin acting via subtype A (IR-A) and B (IR-B) receptors in HUVEC. A metabolic phenotype (p42/44mapk/Akt activity ratio <1) is characteristic of endothelial cells from GDM, an effect that is reversed to a mitogenic phenotype (p42/44mapk/Akt activity ratio >1) by insulin via IR-A in HUVEC. Recent findings show that extracellular adenosine modulates insulin action on L-arginine transport and nitric oxide synthesis in HUVEC via A1 adenosine receptors (A1AR) in GDM but via A2AAR in normal pregnancies.

Financing: Support: VRI+DIDEMUC PUC - Chile. IOR TecMonterrey - Mexico. DIAMATER FAPESP - Brazil.

S24. ROLE OF THE IMMUNE SYSTEM IN HYPERTENSION AND DIABETES

Chairs: Luis Michea (Universidad de Chile, Chile), Kristine Deleon-Pennell (Medical University of South Carolina, USA)

S24-1

Sex differences in T cell mediated hypertension

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Prior to menopause, women are protected against hypertension and its associated cardiovascular complications compared to men, however its incidence and progression are rapidly accelerated in postmenopausal women. The VCD mouse model of menopause is a follicle-deplete, ovary-intact animal that more closely approximates the natural human progression through perimenopause and the postmenopausal stage of life. Our lab has utilized this model to demonstrate that VCD-treated postmenopausal female mice become hyper-responsive to Ang II infusion, displaying a robust increase in blood pressure and cardiovascular dysfunction. The mechanism underlying this shift in blood pressure regulation and disease onset in postmenopausal women is unknown. Postmenopausal women with hypertension do not respond well to current anti-hypertensive medications; 64% of postmenopausal women with hypertension do not have their blood pressure under control.

T cells, an important component of the adaptive immune system, play a critical role in the development of hypertension and cardiovascular disease in males. However, we recently demonstrated that **premenopausal females are protected against T cell-mediated hypertension**. We have demonstrated that protection against T cell mediated hypertension is lost following menopause. Using the VCD model of menopause in T cell deficient mice (Rag1*-/-)* we show that T cell-mediated hypertension progresses rapidly in the absence of ovarian hormones. We will present data to demonstrate that the premenopausal protection against Ang II induced hypertension is mediated via T-regulatory cells. By studying the onset of T cell-mediated hypertension in postmenopausal females, the pathogenic mechanisms uncovered may lead to novel treatments in decreasing hypertension-related complications in postmenopausal females.

Financing: NIH, AHA

S24-2

CD8+ T-cells negatively regulate cardiac tissue biomechanics post-MI

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Introduction: Post-myocardial infarction (MI) left ventricular scar formation and composition is one critical determinant of myocardial remodeling and subsequent declines in cardiac function. CD8+ T-cells are adverse regulators of the post-MI remodeling process leading to decreased cardiac function and survival. **Methods:** To test potential effects on the scar, mice lacking functional CD8+ T-cells ($CD8^{-/-}$) were injected with either vehicle or splenic CD8+ T-cells via tail vein, 4 hours post-coronary artery ligation (Protocol 722 approved by RHJ IACUC). At day 7 post-MI, infarct tissue was collected for passive stretch biomechanical

analysis using an Aurora Scientific tension-length dual mode lever servosystem. The effects of recombinant granzymes (Gzm) B and K on collagen cleavage were tested ex vivo using a fluorogenic collagen cleavage assay. **Results:** Scar biomechanics of $CD8^{-/-}$ mice demonstrated increased regional stiffness compared to WT mice which was lost with splenic CD8+ T-cell re-supplementation. Ex-vivo analysis demonstrated the ability of GzmK to cleave collagen in a concentration and temporal-dependent manner which was not observed with GzmB. Cleavage of the collagen substrate was detected within a 6-hour period at GzmK concentrations as low as 12.5 AU, and as early as 2-hours at 25 AU. GzmK at a concentration of 25 AU demonstrated a 10-fold greater collagen cleavage capacity compared to **Conclusion:** Our data demonstrates that CD8+ T-cells regulate cardiac fibrosis likely through release of granzymes, leading to alterations in the biomechanical capability of the left ventricle.

Financing: This work was supported by the National Institutes of Health T32GM123055; the American Heart Association Innovator Project IPA35260039; the Biomedical Laboratory Research and Development Service of the Veterans Affairs Office of Research and Development Award IK2BX003922 and I01BX003922; and South Carolina Translational Research Center UL1TR001450

S24-3

Antigen-presenting cell modulation of blood pressure

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Introduction: Impaired renal sodium excretion contributes to hypertension, and activating immune responses and chronic inflammation in the kidney may reduce urinary sodium excretion. Dendritic cells (DCs) are professional antigen-presenting cells that orchestrate immunity. In the renal tissue, DCs are present in the interstitium adjacent to the renal tubules and vasculature. Recent studies show that the genetic elimination of DCs or the blockade of DCs function can prevent/lessen the development of hypertension, inflammation, and microvascular remodeling. However, whether renal DCs modulate blood pressure or natriuresis is unclear. **Objectives:** We evaluated if ablation of dendritic cells in mice modified the hypertensive response to AngII, modulating renal sodium handling. **Methods:** We tested AngII or vehicle infusion in cd11c.DOG mice that allowed DC ablation. We analyzed renal DCs, blood pressure (BP), natriuresis, and tubular sodium transport proteins. **Results:** The majority of renal DCs were conventional type-2. The ablation of DCs prevented the increase in BP and the decrease in natriuresis after AngII infusion, which was associated with the modulation of renal sodium-transport proteins. The adoptive transfer of renal myeloid cell fractions enriched in conventional type-2 DCs from AngII-hypertensive mouse kidneys induced a transient increase in BP and reduced natriuresis. In contrast, DCs from normotensive mice or splenic cell fractions enriched in DCs from vehicle or AngII-infused mice did not modify BP or natriuresis. **Conclusions:** These results suggest that renal dendritic cells acquire a pro-hypertensive phenotype after AngII infusion, conferring the ability to modulate renal sodium handling.

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S24-2

Neutrophil gelatinase-associated lipocalin as an immunomodulator in hypertension

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Hypertension is a leading cause of cardiovascular (CV) disease morbidity and mortality. Yet, its pathogenesis is poorly understood and despite the conventional treatments, blood pressure remains uncontrolled in nearly half of the hypertensive patients. Emerging evidence from our group and others implicates immune cells and pro-inflammatory molecules that they produce as pathogenic mediators of this disease and its attendant end-organ damage.

Among these mediators, neutrophil gelatinase–associated lipocalin (NGAL), a secretion glycoprotein of 25kDa, has been closely linked to CV and renal damage in different pathological conditions. NGAL has been proposed as a damage biomarker for target tissues and has also been studied for its role in primary and secondary hypertension. NGAL is produced by many different cell types, can be carried on extracellular vesicles, and is modulated by microRNAs.

Over the last decade, studies have shown that NGAL is necessary not only for salt-sensitive hypertension, but also for the development of aldosterone-induced hypertension that is associated with end-organ damage. In addition, it has been proposed that some mechanisms are dependent on the activation of immune cells, such as dendritic cells and macrophages, where the release of specific cytokines or chemokines would depend on NGAL promoting the subsequent activation of T helper (Th) lymphocytes.

This talk discusses the link between NGAL and hypertension, particularly in the context of aldosterone-induced hypertension and their possible regulators and mechanisms with a focus on its role as an immunomodulator.

Financing: Fondecyt #1201251 and #1231909

S25. HORMONAL SIGNALING IN CARDIOVASCULAR DISEASE

ISHR LAT symposium

Chairs: Celeste Villa-Abrille (CONICET-UNLP, Argentina), Zully Pedrozo (Universidad de Chile, Chile)

S25-1

Adrenocortical hormones and cardiac dysfunction

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Introduction: Glucocorticoid (GR) and mineralocorticoid (MR) receptors are highly expressed in cardiac tissue. Current evidence revealed that MR activation plays an important physiological role to adapt developed force to sudden changes in hemodynamic conditions. However, clinical evidence assigns a detrimental role to MR activation in the pathogenesis of severe cardiac diseases like congestive heart failure, or during acute myocardial infarction. In contrast, the effects of GR activation under pathological conditions are not fully understood, probably because of the controverted scenario generated by the use of different doses of corticosteroids or even compounds with different pharmacological potency.

Objective and Methods: Based on own results obtained from Wistar rats (isolated hearts and/or papillary muscles), the objective of this revision will be to briefly discussing the physiological but potentially pathological consequences of locally triggered cardiac MR signals after myocardial stretch, as well as the functional effects of a low potency glucocorticoid (hydrocortisone) in a rat model of acute myocardial infarction.

Results and Conclusions: Experimental evidence revealed that MR activation after myocardial stretch triggers a previously unknown non-genomic signaling pathway leading to an increase in Ca^{2+} transient amplitude that immediately modulates cardiac contractility, and that acute hydrocortisone treatment during early reperfusion induces cardioprotection against infarction.

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S25-2

Oestrogen signalling as a bridge between the nucleus and mitochondria in cardiovascular diseases

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Introduction: Oestrogen and its metabolites play important but different roles in cardiovascular diseases, depending on the cell type. For instance, in idiopathic pulmonary arterial hypertension (iPAH), elevated oestrogen levels are a risk factor and may control the cancer-like reprogramming observed in pulmonary artery smooth muscle cells

(hPASMC), including mitochondrial dysfunction. However, currently it is not known if 17-β-estradiol or its metabolites are directly able to lead this reprogramming phenotype. In contrast, in cardiomyocytes oestrogen exerts a protective effect that have been described as highly dependent on mitochondrial function.

Objective: To evaluate the effects of 17-β-estradiol and its metabolite, 4-Methoxyestradiol (4-ME), in hPASMC and cardiomyocytes.

Methods: In the context of iPAH, we used a systems biology approach to build a human genome-scale transcriptional regulatory network (TRN) to identify novel transcription factors (TF)-gene interactions and metabolic pathways (MP) affected. We also evaluated whether 17-β-estradiol or 4-ME, modulated the cancer-like reprogramming observed in hPASMC. Parallel, in cardiomyocytes we evaluated the cardioprotective effect of 17-β-estradiol in a model of Norephrine (NA)-induced cardiac hypertrophy.

Results: The MP-integrated TRN showed an estrogenic master pathway in iPAH due to CYP1B1 upregulation. 17-β-estradiol and 4-ME both triggered the cancer-like reprogramming described for hPASMC, including mitochondrial dysfunction. However, in cardiomyocytes 17-β-estradiol exerted a cardioprotective effect front to NA-induced cardiac hypertrophy, which was dependent on the inhibition of mitochondrial fragmentation.

Conclusion: Oestrogens, and particularly 17-β-estradiol, have differential effects in cardiovascular cells that are dependent on the cell type but converge on the regulation of the mitochondrial function.

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S25-3

Cardiac dysfunctional hormonal signalling during menopause

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Estrogen (E) plays a crucial role in women's cardiovascular health, with its decline during menopause increasing cardiovascular risk. A novel player in this context is the G-protein-coupled estrogen receptor (GPER), known for its cardioprotective effects. The renin-angiotensin-aldosterone system (RAAS) is another hormonal system influencing cardiovascular pathophysiology. It has a pressor arm led by Angiotensin II (Ang II) and its AT1 receptor, and a protective arm featuring Ang 1-7, alamandine, AT2 receptor, Mas receptor, and MrgD receptor. E promotes the protective arm while inhibiting the pressor arm of RAAS.

Hormone replacement therapy (HRT), commonly used for menopausal symptoms, doesn't affect RAAS or systolic blood pressure (SBP) and can have adverse effects. Data from our laboratory, using spontaneously hypertensive and ovariectomized rats (SHR-OVX) indicates elevated SBP, worsened cardiac hypertrophy, increased fibrosis, and oxidative stress. Doppler ultrasound revealed diastolic dysfunction with lower papillary muscle compliance.

Conversely, wistar-OVX rats did not show cardiac hypertrophy or fibrosis, with increased GPER expression. In SHR-OVX rats, GPER expression decreased, potentially linked to elevated cardiac Ang II levels. Blocking the angiotensin system in SHR-OVX rats prevented the aforementioned issues and increased GPER expression. The findings suggest that enhancing GPER expression in wistar-OVX rats counteracts cardiac pathologies, whereas in SHR rats with elevated RAAS, decreased GPER expression contributes to cardiovascular disease development and progression. These insights shed light on the complex interplay of hormones and the cardiovascular system in women's health.

S26. HORMONES AND CONTROL OF BREATHING

Chairs: Vsevolod Polotsky (George Washington University, USA)

S26-1

Melanocortins: New Players of Forgotten Players in Control of Breathing?

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Introduction: The melanocortin 4 receptor (MC4R) is a critical regulator of food intake and energy expenditure. An MCR4 agonist setmelanotide is used to treat obesity caused by abnormal melanocortin and leptin signaling. The blockade of the MCR4 signaling has been shown to suppress ventilation and hypercapnic sensitivity. We hypothesized that setmelanotide can treat sleep-disordered breathing in diet induced obese mice and examine mechanisms by which MCR4 may modulate ventilatory control.

Methods: We performed a randomized trial of setmelanotide vs vehicle in obese mice. RNAScope for *Mc4r* and a CO2 sensitivity marker *Phox2b* was performed in the brainstem ventilatory control areas, the nucleus of solitary tract (NTS) and the retrotrapezoid nucleus (RTN), followed by studies with *Cre*-dependent designer receptors exclusively activated by designer drugs (DREADD) in *Mcr4-Cre* mice.

Results: Setmelanotide increased minute ventilation across sleep/wake states, greatly enhanced the hypercapnic ventilatory response (HCVR) and abolished apneas and oxyhemoglobin desaturations during sleep. Setmelanotide also increased oxygen consumption and carbon dioxide production, but ventilation was increased out of proportion to upregulation of metabolism. *Phox2b+* neurons in the NTS and RTN expressed *Mc4r*. Chemogenetic stimulation of the *Mc4r+* neurons in the parafacial region, but not in the NTS, augmented the HCVR without any changes in metabolism.

Conclusion: Setmelanotide treated obesity hypoventilation and sleep apnea in diet-induced obese mice. The effects of setmelanotide are due to increased hypercapnic sensitivity, which likely occurs due to stimulation of *Mc4r*-expressing neurons in the RTN.

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S26-2

Sex hormones and control of breathing

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Breathing is an essential physiological function influenced by numerous factors, including sex hormones testosterone (T), progesterone (P), and estradiol (E2), produced by males and females during adulthood in different concentrations and affect differentially ventilation. Sex hormones have an impact on all aspects of respiratory pathway, from sensory inputs to respiratory brainstem nuclei involved in rhythm and pattern generation up to motor output to the respiratory muscles. In fact, the different respiratory responses in males and females may be associated with the actions of these hormones, especially in adulthood. The fluctuations in gonadal hormones throughout the estrous cycle have also often been identified as significant contributors to the sexual dimorphism observed in ventilation. However, studies from our laboratory observed that endogenous hormonal fluctuations during the estrous cycle were not enough to promote changes in breathing under basal conditions and central and peripheral chemoreflex activation, at least in rats. Interestingly, there is a sex dependence on the prevalence of some respiratory-related diseases, including the panic disorder respiratory subtype, where women have a two to three times higher probability of developing panic disorder (PD) than men during their lifetime. Several studies suggest that individuals with PD exhibit heightened chemosensitivity to CO_2 that potentially triggers panic attacks in these patients. In this lecture, I will discuss the role of sex and gonadal hormones in respiratory control of the locus coeruleus, a sexually dimorphic CO_2/pH-chemosensitive nucleus, on breathing and anxiety behavior in males and females.

Financing: Sao Paulo Research Foundation (FAPESP; 2020/01702-2) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES PrInt; Finance Code 001, Process: 88887.194785/2018-00) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (302991/2022-0)
Acknowledgments: All my previous and current students that collaborate to my research.

S26-3

Stress, orexin, and control of breathing in female rats: insights in the pathophysiology of panic disorders.

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Introduction: Panic disorder (PD) is more frequent in women. An excessive ventilatory response to CO_2 inhalation is a hallmark of PD and this manifestation fluctuates with the menstrual cycle. While the orexinergic system and ovarian hormones contribute to the pathophysiology of PD, their roles remain poorly understood.
Objective: Using neonatal maternal separation (NMS) to induce a "PD-like" respiratory phenotype, we tested the hypothesis that NMS disrupts hormonal regulation of the ventilatory response to CO_2 in female rats. We then determined whether NMS attenuates the inhibitory actions of 17-β estradiol (E_2) on orexin neurons (ORX).
Methods: Pups were exposed to NMS (3h/day; postnatal days 3 to 12). The ventilatory response to CO_2-inhalation was measured across the estrus cycle and following ovariectomy. Expression of ORX1-receptors was quantified in relevant brain regions using *in situ* hybridization. The effect of an ORX_1 antagonist (SB334867; 15 mg/kg) was tested. Excitatory postsynaptic currents (EPSCs) were recorded from ORX neurons using whole-cell patch-clamp.
Results: NMS-related increase in the CO_2 response was observed only when ovaries were functional; the largest ventilation was observed during proestrus. and SB334867 blocked this effect. NMS augmented levels of ORX_A in hypothalamus extracts. Expression of ORX1-receptors varied between brain regions and according to hormonal status. EPSC frequency varied according to basal plasma E_2 levels across the estrus cycle in controls but not NMS.
Conclusion: NMS disrupts the inhibitory actions of E_2 on the respiratory network. Impaired E_2-related inhibition of ORX neurons during proestrus is a novel mechanism in respiratory manifestations of PD in females.
Financing: This research was supported by the Canadian Institutes of Health Research.
Acknowledgments: All protocols and procedures were approved by the U. Laval Animal Care committee in accordance to the guidelines of the Canadian Council of Animal Care.The authors acknowledge the contributions of Dr F Bretzner and M. Henry to this study.

S26-4

Oxytocin and control of breathing

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Clinical studies have shown that oxytocin administered intranasally (IN) decreased the incidence and duration of obstructive events in patients with obstructive sleep apnea (OSA). Although the mechanisms by which oxytocin promotes these beneficial effects are unknown, one possible target of oxytocin could be excitation of tongue-projecting hypoglossal motoneurons in the medulla that exert central control of upper airway patency. This study tested the hypothesis that IN oxytocin enhances tongue muscle activity via excitation of hypoglossal motoneurons projecting to tongue protrudor muscles (PMNs). To test this hypothesis we performed in-vivo and in-vitro electrophysiological studies in C57BL6/J mice as well as fluorescent imaging studies in transgenic mice in which neurons that express oxytocin receptors co-express EYFP. IN oxytocin significantly increased the amplitude of inspiratory-related EMG bursts in tongue muscles. This effect was abolished by severing the medial branch of hypoglossal nerve that innervates the protrudor muscles of the tongue. Oxytocin-

receptor positive neurons were more prevalent in the population of PMNs than in retractor-projecting hypoglossal motoneurons (RMNs). Oxytocin administration increased action potential firing in PMNs, but had no significant effect on firing activity in RMNs. In conclusion, IN oxytocin stimulates respiratory-relating tongue EMG likely acting on central hypoglossal motoneurons that provide tongue protrusion and upper airway opening. This mechanism may play a role in oxytocin-induced reductions in upper airway obstructions in OSA patients.

Financing: NIH grants R01HL128970 and R01HL146169 to DM

S27. VASCULAR DYSFUNCTION WITH COVID-19

Chairs: Shampa Chatterjee (University of Pennsylvania School of Medicine, USA), Amaro Nunes Duarte Neto (Universidade de São Paulo, Brazil)

S27-1

Vascular Transformation: From COVID-19 to Long-COVID

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Long-COVID occurs after SARS-CoV-2 infection and results in diverse, prolonged symptoms. We measured the expression of 3072 plasma proteins with proximity extension assays and then deconvoluted with multiple bioinformatics tools into both cell types and signaling mechanisms, as well as organ specificity. Compared to age- and sex-matched acutely ill COVID-19 inpatients and healthy control subjects, Long-COVID outpatients showed natural killer cell redistribution with a dominant resting phenotype, as opposed to active, and neutrophils that formed extracellular traps. This potential resetting of cell phenotypes was reflected in prospective vascular events mediated by both angiopoietin-1 (ANGPT1) and vascular-endothelial growth factor-A (VEGFA). Several markers (ANGPT1, VEGFA, CCR7, CD56, citrullinated histone 3, elastase) were validated by serological methods in additional patient cohorts. Signaling of transforming growth factor-β1 with probable connections to elevated EP/p300 suggested vascular inflammation and tumor necrosis factor-α driven pathways. In addition, a vascular proliferative state associated with hypoxia inducible factor 1 pathway suggested progression from acute COVID-19 to Long-COVID. The vasculo-proliferative process predicted in Long-COVID might contribute to changes in the organ-specific proteome reflective of neurologic and cardiometabolic dysfunction. Taken together, our findings point to a vasculo-proliferative process in Long-COVID that is likely initiated by either prior hypoxia (localized or systemic) and/or stimulatory factors (i.e., cytokines, chemokines, growth factors, angiotensin, etc). Analyses of the plasma proteome, used as a surrogate for cellular signaling, unveiled potential organ-specific prognostic biomarkers and therapeutic targets. Iosef et al. Journal of Translational Medicine (2023) 21:377. https://doi.org/10.1186/s12967-023-04149-9

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S27-2

Endothelial oxidant signaling post SARS-CoV-2 infection

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Introduction: Recent research suggests that endothelial activation plays a role in COVID-19 pathogenesis by promoting a pro-inflammatory state. However, the mechanism by which the endothelium is activated in COVID-19 is unclear. **Hypothesis**: The "inflammatory load" (cytokine storm) of the systemic circulation causes the activation of endothelial NADPH oxidase 2 (NOX2) that leads to production of reactive oxygen species (ROS) by the pulmonary endothelium. Endothelial ROS activates pro-inflammatory pathways. **Methods**: The inflammatory burden of COVID-19 on the endothelial network, was recreated *in vitro*, by exposing human pulmonary microvascular endothelial cells (HPMVEC) to media supplemented with serum from COVID-19 affected subjects (sera were acquired from patients with COVID-19 infection admitted to the

Intensive Care Unit of the Hospital at the University of Pennsylvania). Endothelial activation was assessed by measuring NOX2 activation (Rac1 translocation) and ROS production in HPMVEC and endothelial inflammation (or appearance of a pro-inflammatory phenotype) was monitored by measuring the induction of intercellular adhesion molecule (ICAM-1) and the NLRP3 inflammasome. **Results:** Endothelial activation and cell death was significantly higher in the COVID-19 model as compared to healthy samples. When HPMVEC were pre-treated with novel peptide PIP-2 that blocks NOX2 activation there was significant abrogation of ROS and endothelial inflammation. **Conclusions**: The endothelium is activated with COVID-19 via cytokine storm driven NOX2-ROS activation which causes a pro-inflammatory phenotype. The concept of endothelial NOX2-ROS production as a unifying pathophysiological axis with COVID-19, raises the possibility of employing PIP-2 as an agent in maintaining vascular health.

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S27-3

Ultrastructural findings in fatal COVID-19: Vessel injury and endothelialitis

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Electron microscopy (EM) continues to play an important role in the pathology of infectious diseases, especially viral diseases, providing important information for diagnosis and insight into pathogenesis. During the COVID-19 pandemic, in its first year in 2020, EM played a key role in understanding tissue invasion by the virus, whose cells were infected by SARS-CoV-2, validating immunohistochemistry and in situ hybridization findings.

Severe SARS-CoV-2 pneumonia causes damage to three compartments of the alveolar parenchyma: epithelial, with infection and direct damage to type II pneumocytes; vascular, with damage to endothelial cells and formation of microthrombi; and interstitial, with induction of viral pneumonitis and progressive deposition of extracellular matrix in the interstitium, causing damage to gas diffusion, with progressive hypoxia in severe cases.

In this session, the main ultrastructural aspects of COVID-19 pneumonia will be presented: 1- Viral particles, rounded or ovoid, with an electron-dense or granular nucleus, with radially distributed spiculations corresponding to the S protein covering the viral nucleus, measuring between 60 and 160 nm, inside dilated endoplasmic reticulum or free in the cytoplasm of the infected cell or in the interstitium; 2 - Detachment of type I epithelial cells from the epithelial layer, vacuolated endoplasmic reticulum, with SARS-CoV-2 virions; 3 - Pulmonary microvasculature showing endothelial damage with rupture of the basal lamina, stacking of blood cells, dysmorphism and hemolysis, fibrin networks and extracellular traps; 4 - Interstitium filled with myofibroblasts and with a large amount of collagen and elastic fibers.

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S28. MOLECULAR AND PHYSIOLOGICAL MECHANISMS OF THE EARLY PROGRAMMING OF DISEASES

Chair: Paola Casanello (Pontificia Universidad Católica de Chile, Chile)

S28-1

Adolescence: Last chance to re-programming health or disease

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Data that support the DOHaD concept comes from early epidemiological studies of exposures in prenatal life. More than 40 years later, the DOHaD concept has been expanded to other plasticity phases of the life cycle, such as preconception, pregnancy, lactation, infancy, and adolescence. Then, any aggression, nutrient restrictions or excess calories, environmental contaminants, or positive factors, such as antioxidants, equilibrated diet, or exercise, among others, can imprint organisms to develop health later in life. Our lab, since 2014, has published a series of 6 papers showing that peri puberty is a programming window. In this current presentation, in the PANAM 2023 meeting, I will share evidence that adolescence can be the last chance to attenuate early programming. The data show that programming imposes changes in puberty, which can determine health fate in adulthood.

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S28-2

Gestational hypothyroxinemia impacts offspring health

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Hypothyroxinemia (HTX) is a condition highly frequent in the first trimester of pregnancy and is clinically characterized by low thyroxine and normal levels of triiodothyronine and thyroid stimulating hormone (TSH). Given that HTX is asymptomatic for the mother, it is harmful to the proper development of the fetus. Both in humans and animal models, gestational HTX at the beginning of pregnancy increases the probability of low capacity for learning and of developing autism spectrum disorder (ASD). Given that this condition is not diagnosed nor treated, our research team has focused on elucidating the cellular and molecular mechanisms that underlie the consequences of HTX in the offspring. Gestational HTX is induced in C57BL6 mice between gestational days E10-E14. The central nervous system (CNS), gastrointestinal system (GIS), and immune system (IS) are investigated at the physiological, cellular, and molecular levels. Inflammatory challenges like autoimmune diseases are induced in the HTX offspring to analyze their immune response. These results support that the consequences of gestational HTX in the offspring surpass the CNS, affecting the GIS and IS. Gestational HTX predisposes an inflammatory state in the offspring and increases the probability of suffering strong autoimmune diseases. This inflammatory state in the offspring could also contribute to the high propensity to develop ASD.

Thus, earlier gestational HTX is a harmful condition for their offspring, and it must be diagnosed and treated to improve the quality of life of their offspring.

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S28-3

Peri-gestational intake of excess added sugars as a risk factor for the early onset of NAFLD in the offspring.

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During the last decades, the concept of the developmental origins of health and disease (DOHaD) has influenced the research on the onset and progression of chronic noncommunicable diseases (NCDs), particularly the worldwide epidemic of metabolic syndrome and their associated comorbidities such as non-alcoholic fatty liver disease (NAFLD). Although originally focused on the outcomes of maternal under- and overnutrition during pregnancy, strong evidence supports that diverse preconceptual, lactational and childhood exposures to environmental and nutritional stressors also contributes to developmental programming. Comorbidities associated with metabolic syndrome have increased globally among children, a fact seemly associated to the consumption of sugar-enriched foods, which correspond to nearly 25% of the total energy intake in childhood. In developing countries, such as Brazil, Chile, and Mexico, over 60% of the population has been reported to consume excessive amounts of added sugars, particularly fructose and sucrose. However, differently from high-fat consumption, the molecular and biochemical mechanisms underlying the metabolic disorders induced by excessive added sugars intake are barely known. Thus, in this conference, I present a body of data generated by our laboratory over the last years on how the peri-gestational consumption of a sucrose-rich diet negatively impacts the liver homeostasis of female rodents and their offspring, promoting an early onset and. progression of NAFLD under both intra- and intergenerational perspectives

Keywords: DOHaD, Metabolic Syndrome, Added Sugars, Steatosis, and NAFLD.
Financing: FAPEMA, CAPES, CNPq.

S28-4

Early programming of obesity in the offspring of women with gestational obesity: insights into mechanisms and opportunity for interventions

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Maternal obesity is the most relevant risk factor for developing childhood obesity and metabolic syndrome in the offspring. The mother's inflammatory and metabolic status can be transferred to the developing embryo and fetus, which can program cell metabolism. In this presentation, a short review of the mechanisms by which maternal obesity can alter the fetal immune function, fetal adiposity and placental function will be described. We set the study at birth and follow-up (4 months) of the offspring of women with pregestational obesity who participated in a randomized controlled trial of Docosahexaenoic acid (DHA) supplementation during pregnancy. The results of this follow-up on neonatal body composition, immune function and methylome, metabolic markers (lipid profile, HOMA-IR, leptin, adiponectin) and placental markers of inflammation and fatty acid transporters will be presented and discussed. The results presented will shed light on our current research project, where we study the possible mechanism of how the obesogenic intrauterine environment could alter the offspring´s progenitor hematopoietic and mesenchymal stem cells, compromising their early cell commitment. These molecular changes are critical to the infant´s programming of the risk of

obesity and chronic diseases and are fundamental to understanding how and when prevention needs to start.

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S29. CHANNELS AND MEMBRANE TRANSPORT IN DISEASES

Chair: Gonzalo Ferreira (Universidad de La República, Uruguay), Luis Sobrevia (Pontificia Universidad Católica de Chile, Chile)

S29-1

Bacterial toxins and heart function: Heat Labile Escherichia coli Enterotoxin B Promotes Changes In CardiacFunction with Possible Relevance for Sudden Cardiac Death.

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Enterotoxigenic *E.coli* secrete enterotoxins, including heat-resistant (ST) or labile (LT). LT is an AB5-type toxin that can bind to specific cell receptors disrupting essential host functions, leading to diarrhea. The pentameric B subunit of LTB binds specifically to certain plasma membrane ganglioside receptors, found in the lipid rafts of cardiomyocytes. We exposed isolated guinea pig hearts and cardiomyocytes to different concentrations of purified LTB fraction. In isolated hearts, we observed cardiac failure with an IC50 of ~0.2 µg/ml LTB, with changes in the patterns of mechanical and electrical alternans, decreased heart rate, and an increment of its variability. In isolated cardiomyocytes, LTB decreased action potential amplitude, and duration. Na^+ currents were inhibited, whereas L-type Ca^{2+} currents were augmented at their peak and fast inactivation. Delayed rectifier K^+ currents were mildly increased. Global intracellular Ca^{2+} concentrations, measured by relative intensity fluorescence by Fluo-3-AM in isolated cells, increased upon exposure to extracellular LTB in resting conditions. This was mostly seen as an increment in the observations of Ca^{2+} release in localized sections of the cell under-stimulation, impairing Ca^{2+} homeostasis. Impaired calcium homeostasis is linked to sudden cardiac death. The results are consistent with the recent view that the B subunit is not merely a carrier of the A subunit, contributing to sudden cardiac death. This is relevant in children (SIDS), infected with enterotoxigenic *E.coli*, explaining several epidemiological findings that establish a strong relationship between SIDS and ETEC *E.coli*.

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S29-2

Role of RyR2 phosphorylation in myocardial stunning/infarction, cardiac arrhythmias and calcium alternans

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Ions, such as calcium (Ca^{2+}), can cross the cardiomyocyte plasma membrane through ionic channels, exchangers, or ATP-dependent pump, as well as through intracellular membranes, e.g., via ryanodine receptors (RyR2). These transport Ca^{2+} from the sarcoplasmic reticulum (SR) to the cardiomyocyte cytosol, providing most of the Ca^{2+} needed for contraction. The precise regulation of these processes impacts the force generated by these cells and is influenced by numerous cytosolic factors and post-translational modifications. The focus will be on the role of RyR2-phosphorylation by CaMKII. Results from our laboratory have shown that Ser2814-RyR2 phosphorylation plays a significant role in CaMKII-dependent irreversible cardiac damage during ischemia-reperfusion. Additionally, in reversible injury due to I/R, we have described that during reperfusion, there is a release of Ca^{2+} from the SR through RyR2, depleting the SR simultaneously with Ser2814-RyR2 phosphorylation by CaMKII. This phosphorylation increases diastolic Ca^{2+} loss leading to membrane depolarization through activation of the electrogenic Na^+/Ca^{2+} exchanger, increasing the susceptibility to reperfusion arrhythmias due to delayed afterdepolarizations. This arrhythmia susceptibility tightly depends on the balance of RyR2/SERCA2a activities. Avoiding

this arrhythmogenic hotspot can lead to the appearance of calcium alternans, a condition that predisposes to arrhythmias and sudden death. In perfused intact mouse hearts, isolated cardiomyocytes, along with a mathematical model of myocytes, we demonstrated that the phosphorylation state of RyR2 modifies susceptibility to calcium alternans. In summary, the phosphorylation state of RyR2 plays a crucial role in the pathogenesis of ischemia-reperfusion, arrhythmias, and calcium alternans, among other disruptions of normal cardiac function.

S29-3

Connexin channels as mediators of cardiac stress-induced arrhythmias and myocardial infarction

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Connexin 43 (Cx43) is the most abundant gap junction channel-forming isoform in cardiac ventricles. In multiple cardiac pathologies, Cx43 is found to be remodeled to the lateralized side of intercalated discs of unhealthy cardiomyocytes. We recently demonstrated that these remodeled Cx43 proteins function as non-junctional channels (hemichannels). The opening of remodeled Cx43 hemichannels mediates cardiac stress-induced arrhythmias and ventricle infarction. We propose that Cx43 hemichannels are an important therapeutic target for the prevention of arrhythmogenic phenotypes displayed in many cardiovascular pathologies.

S29-4

Cellular and Molecular Mechanisms of Mammalian Proprioception

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Proprioception allows for the perception of limb and body position and is required for normal motor output. We recently showed that the sodium channel (Na_V), $Na_V1.1$, is required for proprioceptor coding of sustained muscle stretch and motor behaviors. In addition to $Na_V1.1$, proprioceptors also express Na_V 1.6 and Na_V 1.7. $Na_V1.6$ is required in the brain for motor function, yet its role in proprioception remains unexplored. Currently available genetic tools prevent selective deletion of Na_Vs in proprioceptors; thus, we created a mouse line in which $Na_V1.6$ is removed from all peripheral sensory neurons ($Na_V1.6^{cKO}$). $Na_V1.6^{cKO}$ animals exhibited extreme motor deficits including misdirected hindlimb positioning and an inability to use their tail to guide movement, which we quantified in the open field, grip strength, and rotarod assays. Notably, these motor deficits are distinct from those caused by loss of $Na_V1.1$ in sensory neurons, suggesting that $Na_V1.1$ and $Na_V1.6$ serve discrete and non-redundant roles in proprioceptive signaling. At the cellular level, immunolabeling of muscle spindles show discrete and defined clusters of $Na_V1.6$ at putative heminodes, where it is well positioned to gate signal transmission out of proprioceptor afferent endings. Consistent with this notion, *ex vivo* muscle-nerve electrophysiology showed electrical activity in proprioceptors from $Na_V1.6^{cKO}$ animals was largely abolished. In line with these data, spinal cord electrophysiology experiments found a severely impacted stretch-reflex in $Na_V1.6^{cKO}$ mice. Together, this study provides mechanistic insight into the role of Na_Vs in proprioceptive signaling and behavior.

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S30. CHEMORECEPTORS IN HEALTH AND DISEASE: EXPLORING NEW AVENUES OF TREATMENT

Chairs: Camilo Toledo (Universidad Austral de Chile, Chile)

S30-1

Peripheral chemoreception and the control of breathing

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The carotid body (CB) is the main oxygen sensor that regulates breathing. In the last years, the proposal that the CB contributes to autonomic diseases receive much attention. Obstructive sleep apnea (OSA), featured by chronic intermittent hypoxia (CIH), is considered as an independent risk for hypertension. The pathological cardiorespiratory consequences of OSA have been attributed to systemic oxidative stress, inflammation and sympathetic overflow induced by CIH, but an emerging body of evidence indicates that a nitro-oxidative and pro-inflammatory milieu within the CB is involved in the potentiation of chemosensory responses to hypoxia, which contribute to enhance the sympathetic activity. Accordingly, autonomic and cardiovascular alterations induced by CIH are critically dependent on an abnormally enhanced CB chemosensory input to the nucleus of tractus solitarius (NTS), where second-order neurons project onto the rostral ventrolateral medulla (RVLM), activating pre-sympathetic neurons. CIH produces oxidative stress and neuroinflammation in the NTS and RVLM, which may contribute to the long-term irreversibility of the CIH-induced alterations. In this symposium, I will discuss the contribution of oxidative stress and pro-inflammatory molecules on the hypoxic chemoreflex potentiation including the CB and the brainstem centres, and whether the persistence of autonomic and cardiorespiratory alterations may depend on the glial-related neuroinflammation induced by the enhanced CB chemosensory afferent input of the hypoxic chemoreflex pathway including the CB and the brainstem centres, and if the persistence of autonomic and cardiorespiratory alterations may depend on the glial-related inflammation induced by the enhanced CB chemosensory afferent input.

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S30-2

Central chemoreception and breathing control

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In the last two decades, the search for chemosensitive cells responsible for central respiratory chemoreception has revealed that not only neurons can be chemosensitive, but also glial cells. In fact, now we know that a fraction of astrocytes localized in the rat retrotrapezoid nucleus can release ATP to activate H^+-sensitive neurons in response to acidosis or hypercapnia (high levels of CO_2). In this talk this view will be expanded showing that astrocytes localized in the caudal medulla can also contribute to the regulation of breathing. Medullary astrocytes can release D-serine, a high affinity endogenous agonist for the glycine-binding site of the N-methyl-D-aspartate glutamate receptor (NMDAR), in response to hypercapnia. This property is unique of brainstem astrocytes, and it is not shared by other kind of astrocytes within neocortex and hippocampus. In addition, the hypercapnia-induced ventilatory response in conscious mice appears to depend on the indemnity of microglial population. Preliminary results indicate that the respiratory drive exerted by gliotransmitters is a complex picture. So, a reduction in the influence exerted by one gliotransmitter appears to compromise the effect of a second one.

Our results demonstrate that central chemoreception seems to be, more than a cell-based response, a tissue-based response in which astrocytes, microglia, and neurons are interoceptors.

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S30-3

Altered breathing control in diabetes and the role of chemoreception

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The CBs are peripheral chemoreceptors that classically sense changes in arterial blood O2, CO2 and pH levels. Besides its role in the cardiorespiratory control, the CB has been proposed as a metabolic sensor implicated in the control of energy homeostasis. We found that CB activity is increased in dysmetabolic animal models and in overweight prediabetic patients and that the abolishment of CB activity in animals, via chronic resection of the carotid sinus nerve (CSN), the CB sensitive nerve, or through its electrical neuromodulation, prevents and reverses dysmetabolism as well as sympathoadrenal overactivity, in animal models of metabolic syndrome and type 2 diabetes. In agreement with our pre-clinical data, we have shown that hyperbaric oxygen therapy, an intervention that dramatically reduces CB activity and that is frequently used promote wound healing in diabetic foot, improves glucose homeostasis in type 2 diabetes patients. In this short presentation I will review in a concise manner the role of the CB in the development of metabolic diseases as well as the mechanisms involved in glucose and lipid regulation. I will also debate strategies to adjust CB activity and its reflex responses, which can be used for prevention and treatment of metabolic diseases, envisioning new therapeutic horizons.

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S30-4

Peripheral chemoreceptors and regulation of kidney function in sleep apnea and heart failure

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Sleep apnea (SA) is associated with autonomic dysregulation mediated in part by chronic activation and adaptation of chemoreflex pathways. Enhanced chemoreflex activity is associated with altered efferent sympathetic outflow to renal nerves and related perturbations of renal hemodynamics. Over time renal hemodynamic dysregulation paired with frequent exposure to hypoxemia may lead to tissue hypoxia and injury, and changes in renal function. These changes are relevant to development of chronic kidney disease in patients with obstructive SA as well as progression of renal dysfunction in heart failure patients with central or mixed SA. In this presentation we examine the role of chronic intermittent hypoxia (CIH) associated with SA in regulation of renal artery blood flow, renal microcirculatory perfusion, glomerular filtration rate, and cortical and medullary tissue PO_2 as well as expression of genes that could contribute to renal injury. We also present evidence that the peripheral chemoreflex plays a role in determining renal hemodynamics and tissue oxygenation in chronic heart failure with reduced ejection fraction (CHF). We assessed all physiological parameters at baseline under conditions of normoxia (FiO_2 0.21/$FiCO_2$ 0.00) and subsequently during exposure to 10 bouts of intermittent asphyxia (IA, FiO_2 0.10/$FiCO_2$ 0.03). We found that both CIH and CHF independently contribute to dysregulation of renal hemodynamics and oxygen flux as well as pro-oxidative and pro-fibrotic changes in renal gene expression.

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S31. MYOFILAMENT-BASED MECHANISMS OF MUSCLE DISEASE

Chair: Henk Granzier (University of Arizona, USA)

S31-1

Modulation of cardiac function by cardiac myosin light chain phosphorylation.

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Introduction: The physiological importance of cardiac myosin regulatory light chain (RLC) phosphorylation by its dedicated cardiac myosin

light chain kinase (cMLCK) has been established in humans and mice. Constitutive RLC-phosphorylation, determined by balanced activities of the cMLCK and myosin light chain phosphatase (MLCP), is fundamental to biochemical and physiological properties of myofilaments.

Objectives: We tested the hypothesis that the striated muscle-specific MLCP regulatory subunit, MYPT2, targets the phosphatase catalytic subunit to cardiac myosin and contributes toward the maintenance of cardiac function *in vivo* through regulation of RLC-phosphorylation.

Methods: A floxed-PPP1R12B mouse model was generated to conditionally-ablate MYPT2 in adult cardiomyocytes, and determine its contribution toward RLC-phosphorylation and cardiac function.

Results: We confirmed by immunofluorescence microscopy using the gene-ablated tissue as control that MYPT2 is localized to sarcomeric myosin, and biochemically determined that RLC-phosphorylation increased *in vivo*. Loss of MYPT2 showed a dramatic protection against pressure overload-induced cardiac hypertrophy as confirmed by heart weight, qPCR of hypertrophy-associated genes, measurements of myocyte diameters, and expression of b-MHC protein. Increased ratio of myosin heads distributed to the interfilament space in the MYPT2-ablated heart muscle fibers was measured by mantATP chase assays, confirming RLC-phosphorylation directly regulated by MLCP increases cardiac performance *in vivo*.

Conclusions: Our findings establish MYPT2 as the regulatory subunit of cardiac MLCP distinct from the ubiquitously expressed canonical smooth muscle MLCP. Targeting MYPT2 to increase cardiac RLC-phosphorylation *in vivo* may improve baseline cardiac performance, thereby attenuating pathological hypertrophy.

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S31-2

Roles of myosin-binding protein C in cardiac contraction, disease and therapy

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Myosin-binding protein C (MyBP-C) plays a key role in regulation of striated muscle contraction and relaxation, modulating contractility in the fight-or-flight response, and its mutations cause cardiac and skeletal muscle diseases. With recent success of targeting myofilament proteins, such as myosin, with drugs to treat cardiac diseases, MyBP-C is another attractive target for therapy. However, the mechanisms of MyBP-C function remain incompletely understood, limiting progress to developing new therapies. Recent studies suggest that, in addition to its interactions with myosin, MyBP-C interactions with actin are fundamental to its functions in normal physiology and disease. A critical barrier to studying actin-MyBP-C binding is that conventional methods are time-consuming, involve multiple steps, and have limited throughput. We have developed several biophysical methods using fluorescence lifetime and site-directed spectroscopic probes on actin and MyBP-C that are simple, quantitative, and scalable to better understand these interactions modified by important variables such as phosphorylation, disease mutations, and first-in-class MyBP-C-binding drugs that we have identified. Our mechanistic studies suggest that the mode of binding and protein structures are greatly impacted by PKA-mediated phosphorylation of MyBP-C, the presence of tropomyosin on actin, and missense mutations in MyBP-C causing cardiomyopathy. The development of these assays and improved understanding MyBP-C mechanism enabled us to perform novel high-throughput screens of chemical libraries to identify MyBP-C-binding drugs that increase or decrease its interaction with actin. A subset of these identified drugs also affect myosin ATPase activity in muscle, suggesting their potential development as new medicine to treat contractile dysfunction in cardiomyopathy.

Financing: NIH grants to B.A.C.: R01HL141564, R01AR079435, and 1R43HL162329

S31-3

Myosin Light Chain Mutant-induced Cardiomyopathies

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[1] *University of Miami Miller School of Medicine, Molecular and Cellular Pharmacology, 1600 NW 10th Ave., RMSB 6113, Miami, FL 33136, United States*

Cardiomyopathies, specifically Hypertrophic (HCM) and Dilated (DCM) forms, present distinct types of heart muscle diseases with unique characteristics, etiologies, and clinical manifestations. To elucidate the underlying mechanisms of these diseases, transgenic animal models were developed with HCM-associated D166V or DCM-linked D94A mutations in the myosin regulatory light chain (RLC), which is expressed in heart ventricles. HCM and DCM mice exhibited phenotypic traits consistent with their respective cardiomyopathy. In HCM, left ventricular (LV) and interventricular septum (IVS) hypertrophy, fibrosis, and ultrastructural abnormalities were observed. At the myofilament level, there was increased Ca^{2+}-sensitivity of force and delayed force transients, indicative of abnormal relaxation, supported by the prolonged diastolic index tau, leading to diastolic dysfunction. A shift from myosin's super-relaxed (SRX) state to the disordered relaxed (DRX) state contributed to hypercontractile myosin behavior and pathological cardiac remodeling in HCM mice. Conversely, the DCM-D94A mutation mirrored characteristics of human DCM, displaying LV cavity dilation, structural anomalies, low ejection fraction (EF), reduced cardiac output (CO), stroke volume (SV), and systolic dysfunction. Additionally, DCM-D94A fibers favored the SRX state, suggesting fewer myosin cross-bridges participating in cardiac muscle contraction, potentially explaining compromised in vivo cardiac function in DCM mice. These findings highlight specific changes in sarcomeric structure, function, and myosin energetics in both cardiomyopathies. While their genetic origins are similar, differences in cardiac morphology, function, and molecular-level myosin motor behavior distinguish these conditions. Genetic testing and counseling play a pivotal role in assessing risks and guiding management for affected individuals and families.

Financing: NIH grants: R01-HL071778, R01-HL123255, and R01-HL143830

Acknowledgments: The author expresses gratitude to both current and former members of the Muscle Lab. For more information on our team, please visit: https://lab.szczesna-cordary.miami. Special thanks to our collaborators for their significant contributions and valuable insights into this project.

S31-4

Structure-function relationship of thin filament regulatory proteins in cardiovascular health

J.-P. Jin[1]

[1] *University of Illinois at Chicago, Physiology and Biophysics, 835 S Wolcott Ave, Chicago, USA*

Heart failure is a major medical challenge. Myocardial contractility determines cardiac function. Cardiac muscle contraction is regulated by cytoplasmic Ca^{2+} through the troponin complex in sarcomeric thin filament. The troponin complex consists of three protein subunits: the Ca^{2+} receptor troponin C (TnC), the inhibitory subunit troponin I (TnI), and the tropomyosin-binding subunit troponin T (TnT). While TnC serves as the receptor of Ca^{2+} signal, TnI and TnT are allosteric transducers to transmit Ca^{2+}-induced conformational changes from TnC to activate and deactivate actin-myosin cross bridge cycling. Our molecular evolution and protein structure-function relationship studies have identified submolecular structures of TnI and TnT with functions in modulating myocardial contractile kinetics. Experimental data demonstrate that the C-terminal end 27 amino acid segment of cardiac TnI (C27) is an independently folded peptide structure with a physiological function in selectively reducing the Ca^{2+} sensitivity of activated myofilaments when added to skinned cardiac muscle strips. Consistent with the finding that a C27-like conformation is restored in cardiac TnT upon restrictive N-terminal truncation, the function of C27 peptide mimics the effect of N-terminal truncated cardiac TnT on selectively reducing the end systolic velocity in transgenic mouse heart to sustain stroke volume against afterload. This effect is significantly more potent on hypertrophic cardiomyopathic cardiac muscle, suggesting a novel mechanism to improve cardiac pumping and energetic efficiency in failing hearts. We are now working on translating this troponin-derived regulatory mechanism targeting a specific kinetic step of the contraction-relaxation cycle of cardiac muscle into a new treatment of heart failure.

Financing: Grant HL127691 and HL138007, National Institutes of Health, USA

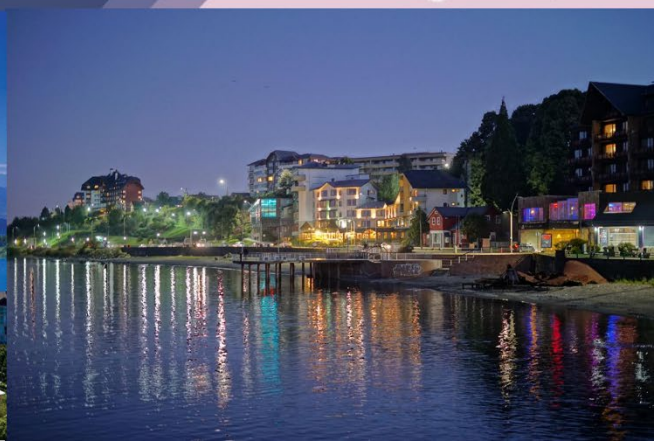
PANAM 2023

Physiological Sciences

Hosted by Chilean Society of Physiological Sciences &
Latin American Association of Physiological Sciences

November 27-30, 2023
Puerto Varas, Chile

Workshops Abstracts



W1. PHYSIOLOGY AND NARRATIVES

Coordinator: Wilson Andrés Parra Chico (Colombia) I/NP/NA

Leonardo Gómez Duarte (Veterinarian) – Universidad Nacional de Colombia NI/NP (neither abstract nor registration)

Iris del Mar Lineros (Physiotherapist) – Universidad Nacional de Colombia NI/NP (neither abstract nor registration)

Wilson Andrés Parra Chico (MD) – Universidad de la Sabana, Colombia NI/NP (neither abstract nor registration)

Carlos Orlando Wilches (Psychologist) – Unigermana Universidad del País Vasco, Spain NI/NP (neither abstract nor registration)

W2. TISSUEGNOSTICS (Sponsored by URSULAB Chile)

Coordinator: Juan Carlos Torres (URSULAB Chile)

W2-1

Exploring the world of tissue cytometry – (Tissuegnostics)

Robert Nica[1]

[1] *TissueGnostics GmbH, Taborstraße 10/2/8 A-1020 Wien, Vienna, Austria*

In-depth profiling of cancer cells and tissues calls for reproducible and robust methods, such as tissue cytometry which combines whole-slide imaging and image analysis. In this workshop, a practical example of tissue cytometry analysis will be shown, where a colorectal cancer sample was stained for seven markers. The aim of this project was to decipher the immune microenvironment through the detection of single cells, in-depth phenotyping of the subpopulations, assessment of epithelium and stroma, and spatial analysis. The contextual image analysis software StrataQuest was used to analyze the sample. The marker-stained cells were identified based on the intensity of the staining. Among the results, number and percentage of marker-positive cells and cell-to-cell contact measurements were performed. For example, out of a total of 1929 CD8+ cells, 880 cells are found in (0-25µm], 428 in (25-50µm], 166 in (50-75µm] and 70 cells in (75-100µm]. All the obtained data can be further used for statistical analysis and data mining for diagnostic, prognostic, and predictive purposes.

W2-2

Tissue Cytometry in neural differentiation and neurodegeneration

Henning Ulrich[1]

[1] *Universidad de Sao Paulo, Institute of Chemistry, Brazil*

Neuronal differentiation, repair processes in the adult brain, neuroinflammation and neurodegeneration are marked by alterations in cellular morphology, proliferation of neural progenitor and glial cells, alterations in the neural and inflammatory expression patterns as well as by induction of cell death. In our work we used stem cells for modeling neurogenesis as well as neurodegenerative diseases as for studying Parkinson's and Alzheimer's disease animal models. We used the Tissuefaxs confocal system (TissueGnostics, Vienna, Austria) for analyzing cellular patterns and neurodegeneration. Overall, tissue cytometry permits quantitative imaging of single cells and tissues without the necessity of removing them from their cellular context.

Financing: This research is funded by the São Paulo Research Foundation and supported by TissueGnostics. H.U. is a scientific adviser of TissueGnostics.

W3. PRECISION IN BREATHING, A WORKSHOP ON SMALL ANIMAL ADULT AND NEONATE PLETHYSMOGRAPHY

Coordinator: Russell Ray (Baylor College of Medicine, Houston, TX, USA)

The aim of this workshop is to cover the recent advances in small animal (cardio-) respiratory measurements while revisiting key fundamentals and best practices that have been, at times, overlooked. Respiratory measurements are being increasingly recognized as important outcome measures in a variety of congenital, neurodegenerative, affective, and infectious disease models that inform upon disease mechanism and clear a path toward therapeutic and diagnostic advances. Poor execution of these techniques will lead to wasted resources, and

erroneous results that misdirect translational efforts. Thus, there is a need in the field to both revisit key fundamentals in breathing studies as well as to highlight novel advances in the field.
In an interchanging presentation, Drs. Cumming and Ray will first discuss the historical development and principles for adult rodent whole-body plethysmography and its elaborations followed by the development, implementation, and principles of neonate pneumotachography. Thereafter, the discussion will move into advances in both adult and neonate rodent measurements and analysis including advanced instrumentation, automation, and data analysis.

W3-1

Precision in breathing, a workshop on small animal adult and neonate plethysmography

Russell Ray[1]

[1] *Baylor College of Medicine, Neuroscience, 1 Baylor Plaza, Houston, United of America*

Financing: United States of America National Institutes of Health grants: R01HL161142 and R01HL130249

Acknowledgments: Everyone in the Ray lab!

W3-2

Precision in breathing, a workshop on small animal adult and neonate plethysmography

Kevin Cummings[1]

[1] *University of Missouri, Biomedical Sciences, Dalton Cardiovascular Research Center, 134 Research Park Drive, Columbia, United States*

Financing: NHLBI grant 5R01HL098602

W4. NEURAL ORBIT (THE NEO PROJECT): IMPLEMENTING NEW PHYSIOLOGICAL TECHNOLOGIES TO MOTIVATE NEW GENERATIONS OF PHYSIOLOGISTS

Coordinators: Alain Riveros-Rivera (Pontificia Universidad Javeriana, Bogotá, Colombia), Tatiana Mendes (ADInstruments do Brasil, São Paulo, Brasil)

W4-1

Neural Orbit (The NEO project): implementing new physiological technologies to motivate new generations of physiologists

Alain Riveros-Rivera[1], Tatiana Mendes[2]

[1] *Pontificia Universidad Javeriana, Physiological Sciences, Medicine, Kr 7 #40-62 Ed.31 Piso 1, Bogotá, Colombia*

[2] *ADInstruments do Brasil, Distribution and Training, Rua Payaguas, No. 10 – Aclimação SP 04109-080, São Paulo, Brasil*

Keeping alive the flame for physiological research is one of the responsibilities of those of us who currently live in this science. For this reason, implementing simple but attractive pedagogical strategies in middle and high school students should be part of our actions. The truth is that most of the time, our efforts are focused on research centers or universities, leaving out the younger ones. This is likely due to the limitations in infrastructure and human and technical resources that a physiology laboratory demands. The aforementioned is even more marked in Latin American countries with tight educational budgets. In this context, the NEO project emerges for mixing space with physiological sciences to bring advanced technology resources to vulnerable student populations in Bogotá-Colombia. This project aims to reduce the gaps in access to technology among the different social classes, allowing low-income students to work with university-level equipment. The objective of this workshop is that participants can perform some practices of the NEO project using portable sensors (Lt sensors technology). These physiological experiments will demonstrate the technology's versatility and how simple demonstrations can motivate students to study physiological and space sciences. As a model, these practices could inspire the teachers in attendance to create their experiments with portable sensors, allowing low-cost experiments to be done in or out-side the laboratory.

Financing: This project has been funded by the Julymar Foundation and Pontificia Universidad Javeriana. ADINSTRUMENTS has sponsored the workshop facilitator's travel and participation.

Acknowledgments: We thank our sponsors and Sabio Caldas and Santa Luisa Schools in Bogotá, including administrative staff, teachers, and students, for their invaluable support. Special gratitude goes to Didaclibros and Mrs. Rosalba Álvarez for their administrative contributions.

W5. BEST PRACTICES FOR PUBLISHING IN THE AMERICAN JOURNAL OF PHYSIOLOGY – RENAL PHYSIOLOGY

Coordinators Luis Michea (Chile) Heddwen Brooks (USA)

W5-1

Heddwen Brooks (Editor-in-Chief AJP-Renal) – Tulane University, USA

W5-2

Publishing in the American Journals of Physiology - Graphs, Figures and ARRIVE Guidelines

Timo Rieg[1,2]

[1] *University of South Florida, Molecular Pharmacology and Physiology, 12901 Bruce B Downs Blvd, Tampa, FL 33612, United States*

[2] *James A. Haley VA, Department of Veterans Affairs, 13000 Bruce B Downs Blvd, Tampa, FL 33612, United States*

Publishing results benefits from clear graphical presentation as well as a detailed enough description of how animal experiments were performed. Regarding the latter, the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines were developed by an international study group composed of researchers from academia and industry, funders, journal editors, and statisticians. The guidelines provide a checklist of recommendations to improve the reporting of research involving animals. The guidelines are a valuable tool to: (i) plan your experiments accordingly, (ii) reflect on which parameters to record during a study in order to provide the necessary information for publication, (iii) ensure that all necessary information is provided when the manuscript is written and (iv) when reviewing manuscripts to determine if all necessary information has been provided. In summary, a clear graphical presentation of results as well as following the ARRIVE guidelines will provide a more uniform reporting of animal experiments which will allow readers and reviewers to analyze the research adequately, draw conclusions on the methodological rigor, and be able to reproduce the methods or findings more easily.

Financing: This work was supported by a VA Merit Review Award IBX004968A. Additional support was provided by a Pilot Project from the USF Microbiomes Institute.

W5-3

Publishing in the American Journals of Physiology - graphical abstracts

Alexander Staruschenko[1]

[1] *University of South Florida, Molecular Pharmacology and Physiology, 560 Channelside Dr., Tampa, USA*

The main goal of a graphical abstract is to give readers a succinct summary of the study, emphasizing key results or ideas. Graphical abstracts quickly and effectively communicate key findings and concepts, making them more accessible to a wider audience, including those who may not be experts in the field. There are many benefits, which are briefly described below:

Attracting Readers: Graphical abstracts can catch the attention of potential readers. A well-designed graphical abstract can serve as a "hook" to attract the interest of readers and draw them into the paper. Researchers are more likely to engage with a paper that has an appealing graphical abstract.

Increased Visibility: Many journals now promote articles on social media and other online platforms. Graphical abstracts are particularly effective in these contexts

Understanding Complex Concepts: Scientific research often deals with complex concepts and data. Graphical abstracts can simplify these complexities and make the main findings and methods more understandable to a diverse audience.

Time-saving: Researchers can save time by quickly assessing whether a paper is relevant to their interests based on the graphical abstract.

Cross-disciplinary Communication: Graphical abstracts can facilitate communication and collaboration between researchers from different fields.

Cultural and Language Diversity: Visual communication is a universal language, which means graphical abstracts can go beyond language barriers.

In summary, graphical abstracts are an invaluable tool for increasing the accessibility, visibility, and impact of research papers, which help to share (for researchers) and to discover and understand (for readers) those findings.

W5-4

Publishing in American Journal of Physiology journals

Luis Michea[1,2]

[1] *American Physiological Society, Associate Editor AJP-Renal, Santiago, Chile*

[2] *Universidad de Chile, ICBM, Facultad de Medicina, Av Independencia 1027, Santiago, Chile*

The editorial process aims to identify manuscripts suitable for publication. The editorial process is a core component of the scientific and academic system, resulting from the collaborative work of authors, editors, and reviewers. This talk aims to describe the main components of the editorial process: the editorial review and the peer review. Starting with the manuscript submission, the talk will discuss the mechanisms of reviewer selection, the duration of each part of the editorial process, and the analysis of the reviewer's assessments and comments by the editors. The mechanism leading to a final decision, accepting or rejecting a manuscript for publication, and communication with the authors will also be discussed. Thus, the participants in the workshop will understand how decisions are made, will have a better understanding of the key elements for preparing manuscripts for submission, and will understand what to expect as a result of the editorial process.

Financing: American Physiological Society

W6. LEVERAGING EDUCATIONAL TECHNOLOGY IN PHYSIOLOGY EDUCATION

Coordinator: Diego F. Niño (Florida International University, Miami, USA)

W6-1

Leveraging educational technology in Physiology education

Diego Niño[1], Stephanie Tadal[1], Jessica Campusano[1], Catarina Jim[1]

[1] *Herbert Wertheim College of Medicine, Florida International University, Medical Education, Miami, USA*

This interactive workshop teaches health sciences educators to create engaging asynchronous instructional materials using free and open-source software. The goal is to provide tools to promote active learning through hands-on experience. Participants will learn to apply educational theories like Technological Pedagogical Content Knowledge, Cognitive Load, and Multimedia Learning to develop effective materials. They will have time to practice new skills with peer collaboration. Small groups ensure high interaction with facilitator guidance.

The workshop addresses the need for educators to integrate educational technologies and evidence-based practices. Participation will enrich learning environments for pre-clinical, clinical, and post-graduate learners. By the end, participants will understand key frameworks and theories, identify applications in their teaching, define active learning and its benefits, summarize cognitive load and multimedia learning principles, and apply these to create instructional materials.

This collaborative workshop facilitates creative problem-solving. Participants gain practical skills to design interactive asynchronous learning experiences grounded in educational theory and technology. The hands-on, interactive format promotes adoption of new approaches to promote active learning. Participants will leave equipped to enrich their instructional environments.

W6-2

Stephanie Tadal (PhD, Director) – Instructional Design & HWCOM, Florida International University, USA NI/NP

W6-3

Jessica Campusano (BS, Instructional designer) – HWCOM, Florida International University, USA NI/NP

W6-4

Catarina Vale (BS, Medical student CO2025) – HWCOM, Florida International University, USA NI/NP

W7. GENERATIVE ARTIFICIAL INTELLIGENCE FOR TEACHERS

(Sponsored by the National Academy of Sciences of Honduras and the Embassy of Honduras to Chile)

Coordinator: Mario Lanza Santamaría (National Academy of Sciences of Honduras, Tegucigalpa, Honduras)

W7-1

Generative Artificial Intelligence for Teachers in Higher Education

Mario R. Lanza Santamaría[1]

[1] *National Academy of Sciences of Honduras, Tegucigalpa, Honduras*

Introduction: This workshop is designed to train educators and higher education experts in the exciting world of generative artificial intelligence (GAI). We will explore how IAG can enrich and transform the teaching and learning experience.

Workshop Content:

1. GIA Fundamentals: We will begin with an introduction to the basics of generative artificial intelligence, including generative neural networks and GAN algorithms.
2. Creation of Educational Content: We will learn how to use the IAG to generate personalized educational materials, such as exercises, exams and multimedia content, which will increase student participation.
3. Interactive Course Design: We will explore how IAG can improve the creation of interactive courses and immersive learning experiences.
4. Feedback and Evaluation: We will address how IAG can automate feedback and evaluation of tasks, allowing teachers to focus on individual guidance.
5. Ethics and Responsibility: We will discuss ethical considerations related to the IAG, including data privacy and equity in education.

Practical application: Participants will have the opportunity to apply the knowledge acquired in case studies and practical projects, adapting the IAG to their own needs in higher education.

Conclusions: This workshop aims to train teachers and higher education experts to use generative artificial intelligence ethically and effectively, thereby improving the quality and personalization of teaching. The IAG not only enriches the learning experience, but also frees up time for individual interaction and guidance, which is essential in higher education.

W8. DIDACTICS OF SCIENCES APPLIED TO THE TEACHING OF PHYSIOLOGICAL SCIENCES

Coordinator: Monica Reinartz Estrada (Universidad Nacional de Colombia, Medellín, Colombia)

W8-1

Some successful teaching methodologies for teaching and learning physiological sciences.

Monica Reinartz[1]

[1] *Universidad Nacional de Colombia, Animal Production, Agrarian sciences, Carrera 53 a 65-110 Robledo, Medellín, Colombia*

In this workshop, two didactic methodologies applied and verified in the animal physiology and neurophysiology courses at the National University of Colombia (Medellín Campus) are shared:

a. Reinartz Student Seminar: based on PBL: of which 30 versions have been carried out nationally and one internationally.

b. Neurodidactic fable: accompanied by the instrument "Neurophysiology notebook", a strategy for students from various areas and professions.

Results: improvement in scientific conceptualization, integration of theory and practice, collaborative work, critical thinking, research discipline. Likewise, it is shown how the application of conceptual change can be used as an evaluative and self-evaluative strategy.

The workshop ends with a workshop on conceptual change and a discussion on the topic between teachers and students from different countries participating in the congress.

Acknowledgments: Universidad Nacional de Colombia.Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, sede Medellín.Organizadores y asistentes del congreso 2023

W9. EXPLORING GAMIFIED PEDAGOGICAL APPROACHES FOR NEUROPHYSIOLOGY IN EDUCATIONAL SPACES

Coordinator: Phoenix Plessas-Azurduy (McGill University, Montreal, Canada)

W9-1

Exploring gamified pedagogical approaches for neurophysiology in educational spaces

Phoenix Plessas-Azurduy[1]

[1] *McGill University, Department of Physiology*

Introduction: The ThinkSci Outreach Program is a workshop-based initiative created to immerse high school seniors and college students into the world of neurophysiology. With a design-thinking approach, gamified to cultivate critical-thinking and problem-solving skills, students get to step into the shoes of neurophysiologists.

Objective(s): We aim to supply opportunities to underrepresented students to experience neurophysiology and empower these students to pursue physiology at the undergraduate and graduate levels/higher education. At PANAM, we hope to empower educators and investigators to bring these tools and approaches to their scientific and educational spaces.

Methods:

1. A brief introduction to the ThinkSci Outreach Program (our mission and goals)
   a. An overview of the structure of our workshops
   b. An introduction to the tools we use and pedagogical approaches we employ. Importantly, the SpikerBox: an affordable and reliable electrophysiological recording tool we use at ThinkSci not only to study neurophysiology at the graduate level but also to teach neurophysiology to a wide variety of ages and educational levels.
2. The bulk of this workshop will be a walk-through of our ThinkSci workshops giving educators the chance to experience our workshops from a learner's perspective
   a. During this portion, attendees will be split into small-collaborative working groups to importantly allow them to experiment with electrophysiological recording (as students would do in workshop)
   b. Additionally, we will periodically interrupt this portion to provide information regarding the pedagogical interventions we choose to employ at ThinkSci to highlight their importance and versatility in application to attendees.

Conclusion (+ Q&A period)

Financing: We would like to thank both the Canadian Association of Neuroscience (CAN) and the Quebec Bio-Imaging Network (QBIN) for funding the ThinkSci Outreach Program.

W10. SCIENTIFIC COMPETITION SESSION IN PHYSIOLOGICAL SCIENCE FOR UNDERGRADUATE STUDENTS PANAM 2023

Coordinator: Ivanita Stefanon (Federal University of Espirito Santo, Vitória, Brazil)

W10-1

Scientific competition session in physiological sciences for undergraduate students

Ivanita Stefanon[1]

[1] *Federal University of Espirito Santo (UFES), Department of Physiological Sciences, Health Sciences Center, Av Marechal Campos, 1468, Maruipe, Espirito Santo, Vitoria, Brazil*

The objective of the Scientific Competition Session in Physiological Sciences is to provide undergraduate students with a platform to showcase their research work and foster academic excellence in the field of physiology. The competition aims to promote scientific inquiry, critical thinking, and effective communication skills among participants. By encouraging active participation and recognizing outstanding achievements, the competition seeks to inspire a passion for physiology research and pave the way for future advancements in the field. Through the presentation, students will have the opportunity to share their findings, methodologies, and conclusions with a panel of evaluators, fostering collaboration and intellectual growth. At the end of the competition, the top three participants will be awarded honorable mentions, acknowledging their exceptional contributions to the field of physiology. The competition is open to all undergraduate students from any recognized educational institution. Six students from those subscribed will be selected to the final oral presentation. Selected students for the final oral presentation will be notified in advance via email. Confirmation of presence is required to secure their participation. Each participant must be the primary author and presenter of the work

submitted in the English language. Students interested in participating must register online by the specified meeting deadline, providing their personal and academic information, along with an abstract of their research work in the English language. The abstract should include a concise summary of the research question, methodology, results, conclusions and financial support. All participants will receive a certificate of participation in the competition.

Financing: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES)
Acknowledgments: PANAM Physiological Sci

TW1. The Journal of Physiology Workshop
Coordinator: Kim E. Barret (EiC The Journal of Physiology, USA)

Journal of Physiology workshop for PANAM 2023 meeting

Kim Barrett[1], Ken O'Halloran[2], Luis Sobrevia[3]
[1] *University of California Davis, Physiology and Membrane Biology, School of Medicine, Sacramento, CA, USA*
[2] *University College Cork, Physiology, Cork, Ireland*
[3] **Pontificia Universidad Católica de Chile, Obstetrics and Gynecology, School of Medicine, Santiago, Chile**

In a workshop aimed predominantly at early career researchers (ECRs), the Editor in Chief, a Senior Editor, and the newly appointed Regional Editor for South and Central America from *The Journal of Physiology* will provide tips on getting your physiological research published, including strategies for writing up your manuscript and how best to appeal to editors and reviewers. The speakers will specifically address the advantages to be gained from submitting your work to *The Journal.* Opportunities for ECRs to get involved with *The Journal* such as our Editorial Board Fellows Scheme and Journal Club articles will also be covered, and there will be plenty of time for questions and answers.

Speakers:

Kim E. Barrett, Editor in Chief, *The Journal of Physiology* and Distinguished Professor of Physiology and Membrane Biology, UC Davis School of Medicine, USA

Ken O'Halloran, Senior Ethics Editor, *The Journal of Physiology* and Professor of Physiology, University College Cork, Ireland.

Luis Sobrevia, Regional Editor for South and Central America, *The Journal of Physiology* and Professor of Molecular Medicine, School of Medicine, Pontificia Universidad Católica de Chile, Chile.

Financing: N/A
Acknowledgments: N/A

TW2. Bentham Science Workshop
Coordinator: Frans Letterström (Director of Global Sales, Bentham Science, United Arab Emirates)

TW3. ELSEVIER Workshop
Coordinator: Rafael Teixeira (Content Acquisition Lead – Biochemistry Journals, ELSEVIER)

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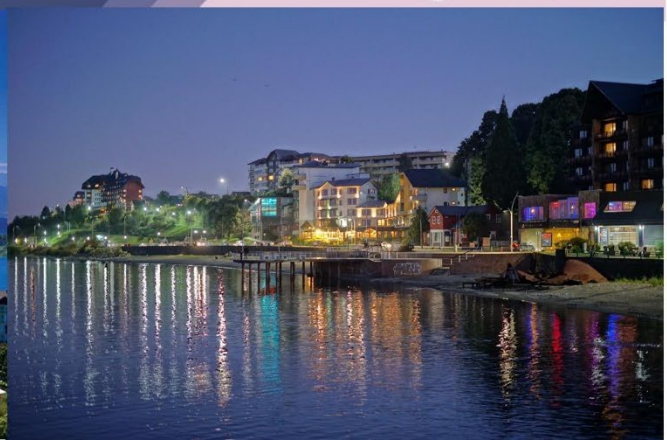

PCC1. SHAPING THE FUTURE OF SKELETAL MUSCLE: METHODOLOGIES AND EMERGING FINDINGS

Coordinators: Denisse Valladares (Universidad de O'Higgins, Chile), Luis Peñailillo (Universidad Andrés Bello, Chile)

PCC1-1

Challenges in the study of DNA methylation

Bernardo J. Krause[1]

[1] *Universidad de O'Higgins, Instituto de Ciencias de la Salud, Alameda 611, Rancagua, Chile*

Epigenetic mechanisms play a key role in physiology, by registering environmental cues and modifying gene expression and phenotypes in the medium and long term. We may consider epigenetics as "the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA sequence" (Cavalli & Heard, 2019), and among them, DNA methylation has been specially studied as a biomarker for the understanding cell and tissue functions. Studies on skeletal muscle physiology have attempted to identify DNA methylation patterns that may account for training history, functional homeostasis, and training responses in subjects with normal and suboptimal skeletal muscle homeostasis. Despite the clear involvement of DNA methylation in regulating gene expression and function, non-conclusive data concerning biomarkers of healthy and/or dysfunctional skeletal muscle has been found. Similarly, scarce support for epigenetic-related biomarkers is reported. In this regard, the lack of consensus on DNA methylation markers for skeletal muscle homeostasis may result from methodological limitations of broadly used DNA methylation profiling approaches. Meta-analysis of DNA methylation data from probe-based arrays supports the low reliability of this type of assay. These issues may come from diverse analytical and data analysis factors, urging the need for more robust approaches. Nonetheless, novel methods for DNA methylation profiling based on real-time sequencing are emerging, in which native DNA and site-specific methylation can be performed.

Financing: Supported by Centro UOH de BioIngeniería (CUBI)

PCC1-2

Impact of skeletal muscle-derived extracellular vesicles and miRNAs

Denisse Valladares[1,2]

[1] *Universidad de O'Higgins, Instituto de Ciencias de la Salud, Rancagua, Chile*

[2] *Centro Interuniversitario de Envejecimiento Saludable RED21993*

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that have emerged as crucial regulators of gene expression and play a pivotal role in maintaining muscle function. miRNAs are instrumental in orchestrating the fine-tuned regulation of myogenesis and muscle growth, influencing muscle protein synthesis, and controlling the balance between muscle hypertrophy and atrophy. Moreover, miRNAs have emerged as critical players in developing muscle atrophy, fibrosis, and insulin resistance. Significantly, they participate in the intricate crosstalk between skeletal muscle and other organs through Muscle-Derived Extracellular Vesicles, often exacerbating systemic diseases like diabetes and cardiovascular disorders.

Understanding the role of miRNAs in skeletal muscle has opened new avenues for potential therapeutic interventions. The ability to manipulate miRNAs offers the potential to mitigate muscle dysfunction in various diseases, enhance muscle regeneration, and improve overall muscle health. We will underscore the pivotal importance of miRNAs in maintaining skeletal muscle function in health and disease. Further research into regulating these small molecules holds great promise for advancing our understanding of muscle-related pathologies and the development of innovative therapeutic strategies.

Financing: This work has been funded in part by Interuniversity Center for Healthy Aging, Code RED211993.

PCC1-3

Applications of High-Resolution Respirometry in skeletal muscle metabolism research

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Abstract: High-resolution respirometry (HRR) using the Oroboros O2k system has emerged as a cutting-edge technique for assessing mitochondrial function and cellular respiration, making it an invaluable tool for understanding

metabolism. This talk will aim to cover the principles, practices, applications, and experiment designs to investigate mitochondrial function, oxidative phosphorylation, and substrate utilization, especially in skeletal muscle permeabilized fibers (PmFB), emphasizing its significance in advancing our understanding of skeletal muscle physiology and metabolism. Finally, we will discuss HRR application in exercise physiology (performance) and non-communicable diseases, addressing respirometry relevance in studying muscle-related disorders and aging effects on mitochondria health.

Financing: Fondecyt Iniciación N°11230186

PCC1-4

Studying mature skeletal muscle fibers: beyond the flexor digitorum brevis

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The fibers isolated from the flexor digitorum brevis (FDB) muscle are a useful model for the study of the biology of the mature skeletal muscle. However, using only the short FDB fibers in muscle experiments carries some limitations, which can be overcome by obtaining fibers from other, longer muscles. Here, we describe how to successfully isolate fibers of different lengths and types from five more hindlimb muscles of adult mice: extensor digitorum longus, soleus, extensor hallucis longus, peroneus longus, peroneus digiti quarti. The fibers isolated from these muscles range between ~1 and ~6 mm and contract briskly for up to 24 h in a saline solution. With the exception of the soleus, these muscles are enriched in fibers type II. We illustrate some potential applications of these long fibers in mature skeletal muscle biology studies.

Financing: CODI-UdeA 2020-34909 and 2021-40170.

PCC1-5

Role of NLRP3 inflammasome in obesity related low-grade inflammation and insulin resistance in skeletal muscle

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Metabolic disorders, such as obesity, insulin resistance (IR), and type 2 diabetes (T2D) are linked to a low-grade but chronic inflammatory state, also known as metabolic inflammation. The precise pathways by which inflammation is triggered and maintained without an overt infection in these pathophysiological states are not fully understood. One current challenge is to find molecular sensors that can respond to environmental cues, such as the nutritional or metabolic status, which trigger the early phases of inflammatory cascades. Current evidence indicates the participation of the nucleotide-binding oligomerization domain-like receptor family (NOD-like) pyrin domain containing 3 (NLRP3) inflammasome in the development of inflammation and insulin resistance in diverse tissues, which are early stages in the pathogenesis of T2D. Currently, limited evidence supports the pathological role of NLRP3 inflammasome activation in glucose handling in the skeletal muscle of obese individuals. We will focus on insulin signaling in skeletal muscle, which is the main site of postprandial glucose disposal in humans. We will be discussing the current evidence showing that the NLRP3 inflammasome disturbs glucose homeostasis. We also review how NLRP3-associated interleukin and its gasdermin D-mediated efflux could affect insulin-dependent intracellular pathways. Finally, we will address pharmacological NLRP3 inhibitors that may have a therapeutical use in obesity-related metabolic alterations.

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PCC1-6

Inflammaging: Implications in obesity and age-related sarcopenia.

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Inflammaging, a portmanteau of "inflammation" and "aging," refers to the chronic, low-grade inflammation that tends to increase with age, contributing to a range of age-related diseases. The mechanisms underlying inflammaging are

intricate and interconnected, with one emerging avenue of research focusing on the association between gut dysbiosis and inflammaging. Gut dysbiosis, the imbalance in the composition and function of the gut microbiota, is increasingly recognized as a pivotal factor in inflammaging. The gut microbiota plays a crucial role in modulating the immune system and maintaining gut barrier function. Alterations in the microbiota composition can lead to a proinflammatory state, with an overabundance of proinflammatory microbial species and reduced diversity. These changes can disrupt the intestinal barrier's integrity, leading to increased gut permeability, allowing microbial products and inflammatory mediators translocation into the systemic circulation, further fueling inflammaging. The gut microbiota's metabolites, such as lipopolysaccharides, can trigger a systemic inflammatory response when they breach the compromised gut barrier. Inflammaging has been linked to various age-related conditions, including sarco-obesity and aging-related sarcopenia. Sarco-obesity refers to concurrent presence of obesity and muscle loss, while sarcopenia is the age-associated loss of muscle mass and function. Inflammaging can promote muscle wasting through the chronic activation of proinflammatory cytokines, which induce muscle protein degradation and impair muscle regeneration. In summary, inflammaging is a critical process in aging, intimately associated with gut dysbiosis, gut barrier function, and permeability. This interconnectedness underscores the importance of understanding the gut-microbiota-immunity axis in the context of aging-related conditions like sarco-obesity and sarcopenia.

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PCC1-7

Fibro-adipogenic progenitors in obese-skeletal muscle

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Excess body fat is a major risk factor for cardiometabolic disease, particularly when ectopically deposited in tissues such as skeletal muscle, known as intermuscular adipose tissue (IMAT). Obesity increases the deposition of IMAT, especially when accompanied by a chronically reduced physical activity level. On the other hand, IMAT aggravates obesity-related muscle metabolic disorders via secretory factors and has been positively correlated with systemic inflammation, impaired glycaemic control and lipid metabolism, and reduced muscle and bone mass in adults with overweight/obesity, thus increasing the risk for the development of type 2 diabetes mellitus. Muscle-resident non-myogenic, mesenchymal fibro/adipogenic precursor cells (FAPs) are recognized as the main cellular components of IMAT. In normal conditions, FAPs participate in the homeostatic maintenance of muscle mass and muscle regeneration, while their fibro-adipogenic potential is inhibited via cross-talk with immune cells, satellite cells and mature muscle fibers. However, studies suggest that obesity-related factors can drive the adipogenic differentiation of FAPs leading to increased IMAT deposition. The aim of this talk in to present data of our ongoing research investigating the association between the length and trajectory of an individual's body mass excess and its related metabolic alterations with the adipogenic differentiation of FAPs and subsequent accumulation of IMAT.

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PCC1-8

Unleashing the benefits of omega-3 lipid mediators in the muscle regeneration process.

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Skeletal muscle is the largest tissue in the human body, comprising approximately 40% of body mass. After damage or injury, a healthy skeletal muscle is often fully regenerated; however, with aging and chronic disease, the regeneration process is usually incomplete, resulting in the formation of fibrotic tissue, infiltration of intermuscular adipose tissue, and loss of muscle mass and strength, leading to a reduction in functional performance and quality of life. Accumulating evidence has shown that the omega-3 (n-3) polyunsaturated fatty acids (PUFAs) and their lipid mediators (i.e., oxylipins and endocannabinoids) have the potential to enhance muscle regeneration by positively modulating the local and systemic inflammatory response to muscle injury. This course will explore how the n-3 PUFAs and their lipid derivatives can positively impact the healing and regeneration of skeletal muscle following injury or damage. The course will

present the newest techniques for conducting research and highlight the emerging evidence in the field.

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PCC1-9

Eccentric exercise in skeletal muscle: good or bad?

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Eccentric exercise is performed when skeletal muscle is actively stretched while producing force (e.g., descending a dumbbell), opposite to concentric exercise in which muscle shortens as it produces force (e.g., lifting a dumbbell). Eccentric exercise is known to induce muscle damage when applied without previous adaptation, but muscle damage can be avoided if correct progression and familiarization are performed. Eccentric training has been shown to induce greater gains in muscle mass and strength, and larger improvements in functional performance and other health outcomes compared to concentric training. Interestingly, eccentric exercise consumes less than 50% of the energy compared to concentric exercise, which translates to lesser cardiometabolic responses to exercise, such as lesser oxygen consumption, and smaller increases in heart rate, blood pressure, and dyspnea. This cardiometabolic efficiency of eccentric contractions may be ideal for clinical populations with exercise intolerance but could benefit from adaptations induced by eccentric training. Furthermore, it has been recently reported that muscle damage and soreness are unnecessary to induce positive adaptations related to eccentric training. In this pre-congress presentation, we will explore the skeletal muscle responses and adaptations to eccentric exercise and its applications.

Financing: This project was funded by FONDECYT #1211962 project.

PCC1-10

It is possible to increase skeletal muscle mass over 85 years old?

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Aging is associated with skeletal muscle mass and function loss. Resistance exercise training can be applied effectively to increase skeletal muscle mass and function in older adults (<85 y). Still, it has been speculated that older adults above 85 y are less responsive to the benefits of resistance exercise training. In this talk it will be demonstrated through an experimental study that prolonged resistance exercise training increases skeletal muscle mass, strength, and physical performance in the aging population, with no differences between 65-75 y versus 85+ y older adults. Consequently, resistance exercise training should be promoted without restriction to support more active, healthy aging, including people over 85 y. Contrary to what has been pointed out by some authors, it is never too late to start with resistance type exercise training.

This study was approved by the scientific ethical committee of Universidad de La Frontera, Temuco, Chile (registration record N°107_18, Folio N°094/18), was performed in accordance with the Declaration of Helsinki and was registered on clinicaltrials.gov as NCT04999501.

Financing: This research was carried out using financial support from ANID - FONDECYT - Chile (Grant Number 11180949).
Acknowledgments: Gabriel Nasri Marzuca-Nassr will present on behalf of all the author's of this study: Gabriel Nasri Marzuca-Nassr, Andrea Alegría-Molina, Yuri SanMartín-Calísto, Macarena Artigas-Arias, Nolberto Huard, Jorge Sapunar, Luis A. Salazar, Lex B. Verdijk, and Luc J.C. van Loon

PCC2. PRE-CONGRESS TEACHING ONE DAY WORKSHOP

(Sponsored by ADInstruments)

Coordinators: Robert G. Carroll (Brody School of Medicine, East Carolina University, USA) Patricia A. Halpin (University of New Hampshire, Department of Life Sciences, USA) Fernanda Klein Marcondes (Dept of Biosciences, Piracicaba Dental School, University of Campinas, Brazil), Dee U. Silverthorn (Dell Medical School, University of Texas at Austin, USA)

PCC2-1

Using active learning methodology for laboratories classes in different models: hybrid, online, or in lab

Tatiana Mendes, Patricia Mendes – ADInstruments ADInstruments

PCC2-2

Basic electronics for physiologists: a tool to create physical manipulatives for teaching purposes

Paulo Montenegro[1]

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Physical manipulatives are concrete objects used as models for a given phenomenon, structure or concept, and may be used as part of active learning strategies in many fields of Science, since they foster hands-on experiential opportunities in the classroom. These models may be created using different materials, and electronic circuits are one of the most amusing ones, because can easily simulate simple physiological stimulus-response pathways.

In this workshop, students will be introduced to the basic concepts of electronics, the most commonly used components, wiring diagrams and online resources on electronic materials, projects and circuit simulation. In a second moment of the course, they will gather in small groups to build simple circuits according to pre-defined diagrams and come up with ideas to use them in manipulative models.

At the end of the workshop, it is expected that the participants will be able to recognize the most common electronic concepts and components, and also build simple circuits as part of physical models to aid learning in physiology. We also expect that participants be encouraged to engage in a continuous learning process in electronics.

Financing: None

PCC2-3

Learning physiology and contributing to the community.

Loreto Pamela Véliz García[1], Carolina Serrano[2], Victoria Velarde A[3]

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Service learning (aprendizaje+servicio, A+S) is a teaching-learning methodology that allows linking the learning objectives of a course into a project that contributes to the community, solving genuine needs in real contexts.

The aims of this workshop are: 1) To work on some strategies that will allow the instructor to visualize how he/she can carry out an activity of A+S in a physiology course. 2) To exemplify the incorporation of reflection and feedback to the social activity developed in the context of the course. 3) To exemplify the development of key transversal skills in students, such as teamwork, oral communication, and social commitment.

In this workshop the participants will be able to work on some strategies that will allow them to visualize how they can carry out an activity of A+S in a physiology course, incorporating reflection, feedback and promoting the development of key transversal skills in students, such as teamwork, oral communication, and social commitment.

For this purpose, the workshop will be divided in an introduction explaining the context of the methodology and the objectives, a reflection activity where participants will work in groups analyzing situations in different learning and social contexts. Then the participants, using the selected learning context, will plan an A+S activity that could be carried out considering one of the learning objectives and/or skills to be developed in a physiology course. Finally, each group will present in a plenary, the work that has been done during the workshop.

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PCC2-4

Publishing your educational scholarship

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Aims of the workshop

- To familiarise attendees with the various formats that manuscript submissions can take

- To support colleagues in creating their next submission
- To enhance chances of manuscript acceptance

Publication of peer-reviewed articles is a meritorious way of increasing scholarly output, gaining international exposure, and is frequently required for career progression of teaching-focused staff. Physiology teachers can publish their innovative teaching methods and teaching-related research in The American Physiological Society journal *Advances in Physiology Education*. This journal offers the optimal platform for publishing scholarly work on teaching and learning of physiology, neuroscience, anatomy and physiology, and pathophysiology, at all educational levels. *Advances* attracts submissions worldwide and has a broad international reading audience because articles are freely available to readers from the time of publication. The workshop facilitators are *Advances* authors, reviewers, and members of the editorial board who will familiarise the participants with the types of articles and the submission and review process. Attendees in small groups will discuss potential educational projects and manuscripts and will have an opportunity to receive feedback on their ideas.

Proposed Structure

- Description of the journal (including the Sourcebook of laboratory activities) - 10 min
- Types of articles/requirements/do's and don'ts - 20 min
- Discussion groups (participant led) on projects and manuscript feedback - 40 min
- Summary remarks (facilitator led) - 20 min

Participation requirements: Participants are asked to bring their ideas for educational research projects or manuscripts in preparation for the discussion groups.

PCC2-5

Smartphone-assisted experimentation for physiology education

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Smartphones are not just a communication technology but an extension of the bodies and minds of the digital-native generation of students. However, a significant amount of faculty does not explore the total capacity of smartphones as a didactic tool. In this workshop, attendees will discuss the pedagogical uses of smartphones to improve and facilitate learning. Ideas and lab protocols will be presented to pave discussions on which smartphones can monitor physiological systems. The principles of inquiry-based learning will be introduced in order to inspire attendees to transform the practical content of a course and engage students as scientists through scientific methodology and creativity.

PCC2-6

Using dramatizations in face-to-face and online courses to teach physiology

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Adding in-class dramatizations to class time is a fun activity in which students act out distinct roles in a 'play' that simulates a physiological process; it has been demonstrated to effectively teach Starling forces, the cardiac cycle, membrane transport, and cell signaling. Dramatizations are inclusive activities for diverse learning styles, such as auditory, visual, and kinesthetic, as each student in the class has a role to play. Students benefit by increasing their confidence level through active participation in an accessible venue that invites them to ask questions and promotes long-term retention of material. The instructor benefits by being able to identify misconceptions and remediate them immediately. Dramatizations can be used in any level of instruction, from undergraduate to professional schools, are free or with minimal costs, and are adaptable to any class size. This workshop will provide participants with the opportunity work in groups to create a dramatization they can use in their own courses. At the end of the session, the participants will have the opportunity to showcase their newly created dramatization and receive feedback from the other attendees. Examples of dramatizations using Zoom to illustrate physiology concepts, which can be used in a lecture, or an asynchronous online course will be shared.

PCC2-7

Using educational games to teach Physiology

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The aim of this workshop is to present examples of educational games (printed and digital) developed to teach Physiology, combined with instructions to promote student engagement, and also with formative assessments. In groups (4 to 6), the participants will receive one education game to solve, and they will analyze and discuss the sequence of activities that are used to provide pre-preparation of students and to evaluate their learning before, during, and after the use of educational games. This workshop includes the educational games: 1) Puzzle of cardiac cycle, and 2) Integrating physiology of synapses, muscle contraction, and autonomic nervous system.

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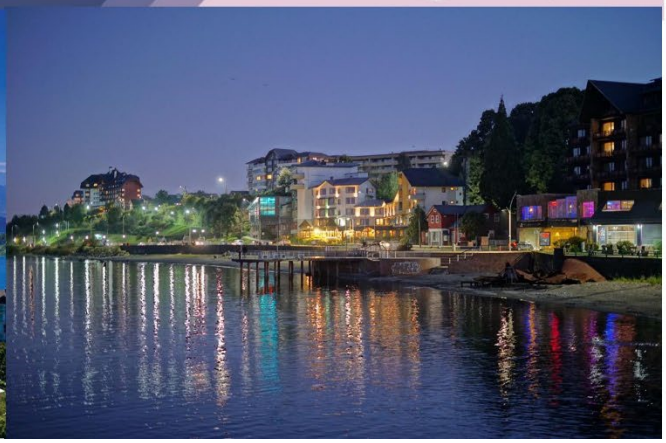

Tuesday 28

AREA: GENERAL

P-1

Performance biomarkers in fish unveiled relevant damage caused by sublethal levels of air-to-water cross-contamination with settleable atmospheric particulate matter (SePM)

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Introduction: SePM pollution has had recent attention due to findings on levels of air-to-water cross-contamination. A new approach employing physiological tools to assess fish physiological performance has emphasized the urgency for risk assessments and emissions control measures.

Methods: We compared Control and SePM-contaminated fish (environmentally relevant; t-test; Ethics Committee#8105110718).

Results: We observed that SePM triggered a clear stress response (cortisol 94% higher). The exposure also reduced hematocrit(12%), hemoglobin (26%), erythrocyte(38%), and mean corpuscular hemoglobin concentration(17%), and concurrently increased mean corpuscular volume(47%) and mean corpuscular hemoglobin concentration(32%). SePM also reduced the respiratory activity of leukocytes(27%) and elevated lysozyme concentration(28%). Leukocytes(43%) and overall plasma protein levels decreased (7%). Recent experiments also indicated that SePM affects gill damage, which compromises respiratory efficiency, increases ventilatory effort, and limits the response to hypoxic conditions. As a result, O2crit was increased(1,31%). Moreover, SePM impacted fish metabolic profile, leading to reductions in standard metabolic rate(26%), maximum metabolic rate(27%), and aerobic scope(29%). This reduced the maximal swimming energetic efficiency(11%).

Conclusion: Therefore, SePM engenders significant detrimental alterations. We observed marked limitations in fish's ability to perform in their environment and cope effectively with routine environmental challenges. Such compromised performance could have far-reaching ecological repercussions and cast implications on broader aspects of fish performance, including long-term energy balance, growth, and reproduction. Hence, SePM contamination may weaken fish, compromising their survival capacity and the general fitness of fish populations. The SePM problem has been largely overlooked by prevailing global monitoring protocols.

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P-2

Early activation of the cardiovascular sympathetic reflex is independent of the occlusion time during reactive hyperemia

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Introduction and Objective: To analyze the role of occlusion time on dynamic changes in autonomic activation during reactive hyperemia.
Methods: Healthy individuals (n=30) aged 18 to 25 years old participated in this study. Vascular reactivity was assessed by measuring the dynamic changes in finger pulse volume amplitude (photoplethysmographic sensors) and pulse transit time relative to the RR intervals (ECG) in the test (occluded arm) and control arm (contralateral non-occluded arm) during 1, 3 and 5 minutes of occlusion. Heart rate variability was calculated. The variables analysis of all signals was displayed every one second in the average response graphics.
Results: Time-varying analysis of the vascular and autonomic response during reactive hyperemia demonstrated the presence of a characteristic response pattern with an increase in the sympathetic index and a decrease in the parasympathetic index between the eight and ten seconds, an increase in heart rate at 20 seconds and a progressive increase in pulse volume amplitude during the first 60 seconds after occlusion; regardless of the time of occlusion.
Conclusion: Early cardiovascular sympathetic activation during reactive hyperemia is independent from occlusion time, suggesting a reflex cardiovascular autonomic response involved in the generation of the physiological phenomenon of reactive hyperemia.

P-3

Autonomic and vascular responses during reactive hyperemia in healthy individuals and patients with sickle cell anemia

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Introduction and Objective: To compare autonomic and vascular responses during reactive hyperemia (RH) between healthy individuals and patients with sickle cell anemia (SCA).
Methods: Eighteen healthy subjects and 24 SCA patients were subjected to arterial occlusion for 3 min at the lower right limb level. The pulse rate variability (PRV) and pulse wave amplitude were measured through photoplethysmography using the Angiodin® PD 3000 device, which was placed on the first finger of the lower right limb 2 min before (Basal) and 2 min after the occlusion. Pulse peak intervals were analyzed using time–frequency (wavelet transform) methods for high-frequency (HF: 0.15–0.4) and low-frequency (LF: 0.04–0.15) bands, and the LF/HF ratio was calculated.
Results: The pulse wave amplitude was higher in healthy subjects compared to SCA patients, at both baseline and post-occlusion ($p < 0.05$). Time–frequency analysis showed that the LF/HF peak in response to the post-occlusion RH test was reached earlier in healthy subjects compared to SCA patients.
Conclusion: Vasodilatory function, as measured by PPG, was lower in SCA patients compared to healthy subjects. Moreover, a cardiovascular autonomic imbalance was present in SCA patients with high sympathetic and low parasympathetic activity in the basal state and a poor response of the sympathetic nervous system to RH. Early cardiovascular sympathetic activation (10 s) and vasodilatory function in response to RH were impaired in SCA patients.

P-4

Feedforward regulation of pentose phosphate pathway

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Introduction: The Pentose Phosphate Pathway (PPP) provides NADPH and diverges from glycolysis. Recent studies suggest that H_2O_2 shifts carbon flux from glycolysis to PPP through a negative feedback regulation by NADPH. Yet, initial exposure to H_2O_2 doesn't disturb NADPH levels, challenging this idea. This might indicate an

anticipatory NADPH production increase. **Objective:** We aimed to determine whether the effects of acute H2O2 exposure are consistent with a feedforward regulation.
Methods: To gather data at high spatial and temporal resolution in intact cells, we used genetically-encoded fluorescent indicators to real-time monitoring relative levels of metabolites such as: glucose (FLII^{12}Pglu-700µδ6), NADPH (Inap1), lactate (Laconic), and pyruvate (Pyronic) in the cell line HEK293. The results were represented by the mean ± SEM with n=3 and were evaluated by t-student.
Results: We observed 2-fold increase in glucose consumption when cells were stressed with 250 µM of H_2O_2. Similarly, levels of pyruvate and lactate were moderately affected under these conditions. Moreover, in the presence of glucose, we didn't observe any decline in NADPH levels at high temporal resolution.
Conclusion: These findings are consistent with the presence of a feedforward mechanism that promptly increase NADPH production when the cells are under stress. Also, there is no clear redirection of the carbon flux from glycolysis to the PPP. Instead, the feedforward regulation seems to involve redirection at glucose-6-phosphate level.

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P-5

Zoledronic acid inhibits the activity of the Transient Receptor Potential Vanilloid Type 1 (TRPV1)

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Introduction: The TRPV1 channel, is the molecular substrate that initiates pain responses. Molecules targeting the TRPV1 channel represent a potential therapeutic avenue for pain relief, inflammation and burning sensation. We have obtained evidence that the bisphosphonate zoledronic acid (ZOL) acts as an inhibitor of the TRPV1 channel. ZOL is used as a treatment for bone disease, reducing bone loss and is not classified as an analgesic.
Objective: To determine the molecular mechanism by which zoledronic acid inhibits TRPV1 channel activity.
Methods: By expressing TRPV1 in *Xenopus laevis* oocytes (N° BEA125-18, CIBICA-UV) followed by inside-out patch-clamp recordings, single-channel (mean + S.E.M: 2.06 ± 0.13; 0.82 ± 0.04; N=5-6), and macroscopic currents (Tau 7.1 s for 10 nM ZOL and 3.8 s under 30 µM ZOL; N=3-4), site-directed mutations (1 ± 0.14; 0.2 ± 0.8; N=1; 0.91 ± 0.12; 0.3 ± 0.07; N=1) and molecular dynamics.
Results: ZOL at doses ranging from 1 nM to 100 µM reduces channel conductance and deeply inhibits channel activity. Only high doses of CAP (≥ 10 µM) allow recovery of TRPV1 currents after ZOL treatment. Molecular modeling followed by docking analysis suggests that ZOL could potentially bind TRPV1 at the vanilloid pocket, the $PI(4,5)P_2$ binding site, and a site located around the ankyrin repeat domains. Ongoing experiments with neutralizing mutants of the $PI(4,5)P_2$ binding

site seem to indicate that this pocket is only weakly involved in the binding of ZOL to the channel.

Conclusion. Taking together, our evidence strongly supports that ZOL can inhibit TRPV1 activity.

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P-6

eNOS localization mediates inactivation of inflammation-induced hyperpermeability

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Introduction: Hyperpermeability is the hallmark of inflammation. However, the consequences are due to impairment in restoring normal permeability, so we are studying mechanisms that terminate hyperpermeability.

Objective: We tested if the agonist signaling for hyperpermeability (endothelial nitric oxide (NO) synthase (eNOS) internalization to cytosol) also initiates its inactivation by a delayed increase of cAMP concentration ([cAMP]), causing translocation of eNOS and Epac1 from the cytosol to the cell membrane.

Methods: We study hyperpermeability restoration induced by vascular endothelial growth factor (VEGF) in vivo with mice mesentery vessels and in vitro using human microvascular endothelial cells (HMVEC) and ECV-304 cells that stably express cDNA constructs targeting eNOS to the cytosol or plasma membrane. Data were shown as mean ± SEM and n=5. We used one-way ANOVA test. All animal experiments were approved by the IACUC of Rutgers New Jersey Medical School (PROTO999900759).

Results: VEGF-stimulated HMVEC or mice mesentery vessels exhibit increased hyperpermeability. However, Epac1 stimulation, after application of VEGF, inactivates hyperpermeability. Interestingly, when eNOS is targeted in the cytosol or the membrane, VEGF-induced hyperpermeability is unaffected after Epac1 stimulation. VEGF stimulates a delayed increase in [cAMP] in HMVEC and is blunted by eNOS inhibition with a global NOS inhibitor or when eNOS was anchored to the cytosol or the membrane. eNOS and Epac1 are closely located at the times when [cAMP] increase is detected in HMVEC.

Conclusion: Our results demonstrate that eNOS, cAMP and Epac1 work in synchrony to terminate hyperpermeability by translocating eNOS and Epac1 back to cell membrane.

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P-7

Water physiology: Impact of torpor on water balance in a marsupial (Thylamys elegans) from a dry mediterranean environment

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Introduction: Torpor enables energy conservation under unfavorable conditions for maintaining energy balance, such as extreme temperatures and food scarcity. Additionally, certain studies on marsupials from mediterranean environments suggest that could contribute to maintaining body's water balance by reducing evaporative water loss (EWL). However, there are currently few studies on marsupials from water-scarce environments.

Objective: We analyzed the relationship between torpor and water conservation in *Thylamys elegans* from a dry mediterranean environment.

Methods: Fourteen individuals were measured using indirect calorimetry to assess resting metabolic rate (RMR) at 30°C and 15°C after a 24-hour fast. We estimated metabolic water production (MWP), measured body temperature (Tb) and employed a hygrometer to measure EWL. We used paired t-test as statistical analysis for comparisons. This study was approved by Bioethics Committee of Universidad of Chile (No 1914-FCS-UCH/2019) and Agricultural Livestock Service (No 2894/2019).

Results: The animals exhibited torpor and hypothermia (Tb = 18 ± 1°C, mean ± SD) at 15°C, while at 30°C, they remained normothermic (Tb = 32 ± 1°C, mean ± SD). Compared with normothermia, during torpor RMR, MWP and EWL decreased significantly ($p<0.05$) by 75%, 75% and 40%, respectively. Meanwhile, in torpor the relative water economy (MWP/EWL) decreased by 60%.

Conclusion: We conclude that torpor reduces water loss but does not favor water balance because the depression in metabolic rate leads to a more significant reduction in MWP than EWL. We predict that sopor is less favorable for sustaining water balance in water-scarce environments than under normothermic conditions but could help endure water scarcity.

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P-8

H2S is involved in the regulation of intracellular pH in human umbilical vein endothelial cells from healthy and gestational diabetes mellitus pregnancies

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Introduction: Intracellular pH (pHi) in human umbilical vein endothelial cells (HUVECs) from gestational diabetes mellitus (GDM) pregnancies is regulated by the Na^+/H^+ exchanger 1 (NHE1). NHE1 activity is inhibited by hydrogen sulfide (H_2S) and synthetized by cystathionine gamma-lyase (CSE).

Objective: To determine if endogenous or exogenous H_2S regulate the NHEs activity and pHi in HUVECs from heathy and GDM pregnancies.

Methods: HUVECs were isolated from anonymous healthy (n=6) and GDM (n=4) pregnancies at UMCG (UMCG, U Groningen, NL, patient consent, Ethics N°RR10959). HUVECs (passage 3) were exposed to 0-1000 µmol/L sodium hydrosulfide (NaHS, 30 min, H_2S donor) or propargylglycine (PAG, 24 h, CSE inhibitor). pHi was measured in cells preloaded with fluorescent pH-sensitive probe BCECF-AM (12 µmol/L, 10 min) and exposed to NH_4Cl (20 mmol/L). The pHi recovery rate (*dpHi/dt*) were estimated in cells exposed to 5 µmol/L 5-N,N-hexamethylene-amiloride (HMA, NHEs general inhibitor).

Results: Basal pHi was higher ($P<0.05$, two way-ANOVA, mean±SEM) in GDM compared to healthy pregnancies (pHi 7.4±0.1 and 7.7±0.1, respectively). Basal pHi was reduced with NaHS (pHi 7.4±0.1 and 7.1±0.1, respectively) but unaltered by PAG. GDM pregnancies showed no changes in basal pHi. *dpHi/dt* was reduced by 56±15% with NaHS but unaltered by PAG in healthy pregnancies. In GDM, *dpHi/dt* was

reduced by 94 and 46% with NaHS and PAG, respectively. NHEs-mediated *dpHi/dt* shown similar values as NaHS in healthy and GDM pregnancies.

Conclusion: pHi is regulated by H_2S in an NHE-dependent manner, in healthy and GDM pregnancies, whereas CSE-synthesized endogenously-H_2S did not affect pHi.

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P-9

Hypoxia-responsive genes and COVID-19-related neuroinflammation: A comparative gene expression study

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Introduction: COVID-19 is a systemic disease that causes neurological symptoms during acute phase. The processes of neuroinflammation, hypoxia, and microglial activation are interrelated in various central nervous system (CNS) disorders. Recent findings indicate that Hypoxia-inducible factor-1(HIF-1) may drive neuroinflammation, and blood-brain barrier disruption in COVID-19-related brain damage.

Objectives: In this study, we investigated whether the expression of hypoxia-responsive genes would correlate with alterations in the expression of neuroinflammatory cytokines in different brain regions during acute COVID-19.

Methods: The animal experiments were approved by the CEUA at the Health Sciences Center/UFRJ, following international research animal standards (Approval No. 106/21).. We conducted a comparative gene expression analysis of proinflammatory cytokines (*TNF-α, IL-1β, and IL-6*) and hypoxia-responsive genes (*Hif1a, Vegf, Hmox*) in response to SARS-CoV-2 infection in K18-hAce2 mice. Statistical analysis utilized unpaired two-tailed Student's t-tests and one-way ANOVA with post-hoc Bonferroni tests, employing Mean ± standard deviation (S.D.) values. The control group comprised 5 animals, while the infected group included 15 animals.

Results: SARS-CoV-2 infection (4dpi) induced the upregulation of proinflammatory cytokines in the cortex and hippocampus. The gene expression alterations varied between the brain regions analyzed. Surprisingly, SARS-CoV-2 infection caused a ~2-fold decrease in *Hif1a* mRNA expression in the cortex and hippocampus, while other hypoxia-responsive genes remained unchanged. Iba-1-positive cells underwent morphological alterations in infected mice, indicating microglial activation.

Conclusions: These findings suggest that microglial activation mediates a brain region-specific upregulation of proinflammatory cytokines, potentially independent of hypoxia-responsive genes in the K18-hAce2 model of acute COVID-19.

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AREA: CARDIOVASCULAR AND RESPIRATORY

P-10

Effects of the hepatokine LEAP-2 on cardiomyocyte hypertrophy

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is an independent risk factor for cardiovascular diseases. Besides hepatocyte lipid accumulation, NAFLD involves changes in hepatic secretion of lipids and hepatokines. Previous RNA-seq studies from our group, identified the liver

expressed antimicrobial peptide 2 (LEAP-2), the endogenous antagonist and inverse agonist of ghrelin; as the most increased hepatokine in NAFLD. Interestingly, ghrelin had demonstrated cardioprotection in different hypertrophy models through a PGC1α-dependent pathway. However, the role of LEAP-2 on cardiomyocyte hypertrophy remains unexplored.

Objective: To elucidate the effects of LEAP-2 on cardiomyocyte hypertrophy.

Methods: Experiments were carried out according to our institutional ethics committee (CICUA-CQyF-2023-52). Neonatal rat ventricular cardiomyocytes (NRVCM) were treated with LEAP-2 and ghrelin at increasing concentrations (10 nM - 1 µM) for 48 hours to evaluate hypertrophic markers mRNA levels. Norepinephine (NA) was used as positive hypertrophy control. Data is shown as mean ± SEM (N = 4-5) and analyzed by one-way ANOVA with Dunnett's multiple comparison test.

Results: LEAP-2 (1 µM) treatment of NRVCM increased the mRNA expression of the hypertrophic marker ANP (3.1±0.69, N=5, $p<0.05$) without changes in BNP, β-MHC, or RCAN1.4; as well as downregulated GLUT4 mRNA (0.48±0.09, N=5, $p<0.05$) without changes in GLUT1. PGC1α mRNA levels were unaltered. On the other side, ghrelin 1 µM did not change hypertrophic markers, but increase PGC1α mRNA levels (1.7±0.29, N=5, $p<0.05$).

Conclusion: LEAP-2 increases cardiomyocyte mRNA expression of hypertrophic markers ANP and GLUT4 regardless PGC1α. Further studies are required to understand the hypertrophic molecular mechanism of LEAP-2 signaling.

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P-11

Cardiac dysfunction during unloading-induced atrophy. Relation with BIN expression as a biomarker of cardiac health

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Introduction: Unloading during prolonged bed rest, sedentary lifestyle or microgravity induces cardiac atrophy, associated to dysfunction through T-tubules (TT) disorganization. BIN1 is a crucial protein to maintain cardiac TT structure and heart function. Besides, BIN1 expression can be decreased by c-Myc in cancer cells, however, it is unknown whether BIN1 levels changes during unloading and its association to c-Myc in cardiac tissue.

Objective: To determine BIN1 and c-Myc expression during cardiac atrophy induced by hemodynamic unloading in a murine preclinical model.

Methods: Unloading of the body was induced in male C57BL/6 mice by hindlimb suspension (HSU) for 14 days (FMUCH#23672). Cardiac function was measured by echocardiography and heart weight was obtained as a atrophy parameter. Markers of remodeling were measured by qRT-PCR and BIN1 and c-Myc expression were measured by Western blot and qRT-PCR, respectively. Student's t test or one-way ANOVA and Tukey's post-test were used for statistical analysis and data were expressed as the mean ± SEM (n=5-8).

Results: Decreased ejection fraction and fractional shortening was developed in HSU mice indicating deteriorated cardiac function, associated to diminished heart weight. mRNA of β-MHC, BNP, ANP and the reason Col1/Col3 were increased after HSU in cardiac tissue. BIN1 protein and mRNA levels decreased while c-Myc increased in HSU mice respect to the controls.

Conclusion: Cardiac atrophy, remodeling and dysfunction induced by HSU are associated to decreased BIN1 expression and increased c-Myc levels, suggesting that BIN1 could at least in part, explain the cardiac contractile dysfunction during atrophy induced by unloading.

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P-12

Compounds from Senecio nutans reduce the intracellular calcium in vascular smooth muscle cells (A7r5) and primary cultured rat aorta

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Introduction: Hypertension is a relevant health challenge in Chile, affecting 27.6% of the population and being a significant cardiovascular risk factor. In this context, the endemic plant *Senecio nutans* Sch. Bip (chachacoma) is used in traditional medicine to reduce blood pressure. Metabolites have a vasodilator effect, but the role of intracellular calcium ($[Ca^{2+}]i$) is still unknown.

Objective: To compare the effect of compounds from *Senecio nutans* on the intracellular calcium handling in A7r5 cells and primary cultured rat aorta.

Methods: The compounds studied were 4-hydroxy-3-(3-methyl-2-butenyl) acetophenone (SGI), 5-acetyl-6-hydroxy-2-isopropenyl-2,3-dihydro benzofurane (SGIV), and their oximes. To evaluate the intracellular calcium levels, KCl (50 mM) was used to depolarize the plasma membrane in vascular smooth muscle cells (A7r5). Cells were loaded with Fluo4-AM (10 µM), to determine changes in the intracellular calcium by using confocal microscopy. Two-way ANOVA and post-hoc Bonferroni test were used. *p <0.05 (data as mean ± SEM, n=5). Ethics Committee of Universidad de Antofagasta (CEIC-275/20).

Results: All compounds reduced the $[Ca^{2+}]i$ in A7r5 cells in response to KCl. The SGI compound showed a lower increase in $[Ca^{2+}]i$ compared to the SGI oxime, which exhibited a significantly greater increase with the KCl stimulus. SGIV displayed a greater increase in $[Ca^{2+}]i$ compared to the SGIV oxime. The primary culture results were consistent with the effects observed in the A7r5 culture.

Conclusion: Compounds isolated from *Senecio nutans* could have significant pharmacological potential in regulating calcium homeostasis in vascular smooth muscle cells. Notably, the metabolites SGI and SGIV oxime emerge as possible candidates for the treatment of hypertension.

Financing: Financial support was provided by FONDECYT 1200610 to J.P.

P-13

Cholinergic stimulation reduces the platelet adhesion to endothelial cells and the expression of adhesion proteins during endotoxic condition

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Introduction: Sepsis, a life-threatening condition with high mortality, often leads to coagulopathy in over 50% of patients. Endothelium, a major actor in hemostasis regulation, becomes highly adhesive to platelets during sepsis by means of increasing the expression of adhesion molecules VCAM-1, ICAM-1, and P/E selectins. It is suggested that cholinergic stimulation decreases platelet adhesion to endothelial cells (EC). However, the impact of cholinergic stimulation on pro-adhesive phenotype of EC under septic condition is poorly studied.

Objective: To determine the impact of cholinergic stimulation role in reducing platelet adhesion to EC and endothelial adhesion proteins expression under endotoxic conditions.

Methods: EA hy.926 cells were exposed to lipopolysaccharide (LPS) or vehicle for 24h and pretreated 1h before LPS exposure with the cholinergic receptor agonist GTS-21 or the antagonist MLA. Gene expression of adhesion proteins was determined via RT-qPCR 24h after LPS exposure. Platelets were purified independently from blood of healthy volunteers (protocols were approved by the institutional Bioethics Committee of the Universidad Andrés Bello resolution N° 002/2020). After 4h of LPS exposure, ECs were co-cultured with platelets for 20h. Adhesion was evaluated through fluorescent microscopy. Results are presented as mean±SD (N=4) and analyzed by one-way ANOVA and Tukey's *post hoc* test.

Results: LPS increased platelet adhesion to EC 4 folds relative to control. GTS-21 pretreatment reduced platelet adhesion to control levels. This effect was prevented in the MLA-treated groups. Adhesion molecules mRNA expression showed similar trend in the different conditions.

Conclusion: Cholinergic stimulation reduces endothelial pro-adhesive features reducing the endothelial adhesion molecules expression.
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P-14

A new chalcone synthetized from Senecio nutans metabolite reduces vasoconstriction through blocking voltage-gated L-type calcium channel (CaV1.2) in spontaneously hypertensive rats (SHR)

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Introduction: The chalcone family has attracted much interest due to its potential antihypertensive activities. Here, we have synthesized a new chalcone (CHAL13) from 4-hydroxy-3-(3-methyl-2-butenyl) acetophenone, a metabolite present in *Senecio nutans* Sch.Bip., and 2,4,5-trimethoxybenzaldehyde.

Objective: To study the mechanism of action of a new chalcone on vascular smooth muscle in the aorta of spontaneously hypertensive rats (SHR).

Methods: The vascular effect of CHAL13 was evaluated in phenylephrine- and KCl-induced dose-response contraction curves in aortic rings. Calcium influx was studied in Ca^{2+}-free medium. The effect of CHAL13 (10^{-5} M) on intracellular Ca^{2+} levels was evaluated in primary culture of vascular smooth muscle cells (pVSMC) loaded with Fluo4-AM (5µM). Results represent mean ± S.E.M (n=4-6) and were analyzed by two-way ANOVA and Bonferroni test ($p<0.05$). Ethics Committee of Universidad de Antofagasta (CEIC-275/20).

Results: In the presence of CHAL13 (10^{-5} M), the contractile response to phenylephrine (70.2±12.6% versus 109±6% control) and KCl (54.8±6.3% versus 94.5±7.3% control), was reduced. CHAL13 reduced the KCl-induced increase of intracellular calcium (Fluo4-AM fluorescence) in aortic pVSMC from SHR. Preincubation with CHAL13 (10^{-5} M) significantly reduced (62.2±9.7% versus 110±13% control, $p<0.001$) the vascular contraction to Bay-K8644 (10 nM; a $Ca_V1.2$ channel agonist), and significantly ($p<0.05$) decreased (62±10.6% versus 96.3±3.3%) the contraction induced by calcium influx in a Ca^{2+}-free medium, suggesting the involvement of the voltage-gated L-type calcium channel ($Ca_V1.2$).

Conclusion: A new chalcone synthetized from *Senecio nutans* metabolite reduces vascular contractility, this is mediated through blocking of the voltage-gated L-type calcium channel $Ca_V1.2$ in the aorta of spontaneously hypertensive rats (SHR).

Financing: Financial support was provided by FONDECYT 1200610 to J.P.

P-15

Contribution of COX/TXA2 pathway on the vascular reactivity mediated by male sex hormones

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Introduction: Testosterone exerts cardiovascular effects through genomic and non-genomic mechanisms. While its acute vasodilatory action on vascular smooth muscle function has been established, its long-term impact on vascular reactivity remains unclear.

Objective: We investigated the hypothesis that testosterone participates in the long-term regulation of vascular tone via the cyclooxygenase/thromboxane (COX/TXA2) pathway.

Methods: Male Wistar rats were divided into orchidectomized (OQT, N=11) and control (SHAM, N=11) groups. After 12 weeks, the aortic rings were isolated for the assessment of vascular reactivity to phenylephrine (10^{-11} to $10^{-3.5}$ M) in the

presence of inhibitors: Indomethacin 10 µM (non-selective COX inhibitor), NS 398 1 µM (selective COX2 inhibitor), SQ 29.548 1 µM (selective thromboxane receptor blocker), and furegrelate 10 µM (TXA2 synthase inhibitor). Ethical approval (CEUA-UFES 17-2020). Statistical analysis: Student's t-test or one-way ANOVA, significant to P<0.05.

Results: The Rmax to phenylephrine was similar between control groups (SHAM:118.1±6.29, N=7; OQT:115.9±4.38, P>0.05, N=13). Furegrelate equally reduced Rmax in both groups (SQ 29,548; SHAM: 67.1±3.20*, N=9; OQT: 77.12±3.7*, N=8, *P<0.05). The vascular reactivity was not affected by indomethacin, NS 398, and furegrelate in either group: Indomethacin (SHAM:99.8±2.8, N=6; OQT:119.4±2.84, P>0.05, N=9), NS 398 (SHAM:101±4, N=6; OQT:97.1±2.4, P>0.05, N=8), and furegrelate (SHAM:107.9±4.78, N=8; OQT:110.8±3.9, P>0.05, N=11). The results demonstrated that phenylephrine-mediated vasoconstriction was equally dependent on TXA2 receptors in the SHAM and OQT groups.

Conclusion: We conclude that testosterone, in the long term, does not appear to participate in the regulation of vascular reactivity via the COX-TXA2 pathway in isolated aortic rings of young rats.

Financing: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (Fapes), CNPq, UFES

P-16

Evaluation of natural compounds from bees (Propolis and Apitoxin) and some of their purified components (Pinobanksine and Melittin) on basal cardiac function and on ischemia-reperfusion injury

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Introduction: Propolis and Apitoxin are mixtures of many components with a high Oxygen Radical Absorption Capacity index. This is primarily attributed to polyphenols/flavonoids in Propolis and polyamines in Apitoxin.

Objective: Considering that cardiac metabolism is critically dependent on mitochondria (a key source of reactive oxygen species), our study aimed to assess their impact as complete mixtures or individual components (Pinobanksine and Melittin) on basal cardiac function and ischemia-reperfusion injury (IRI).

Methods: All studies were performed under ethical approval (07015150021221; 07015100003022). Isolated guinea pig hearts were retroperfused through the coronaries with 1.8 mM Ca^{2+} Tyrode, recording simultaneously mechanical and electrical responses.

Results: Propolis between 0.006% and 0.01% promoted positive inotropism; whereas beyond 0.02% promoted negative inotropism, having a bell-shaped or biphasic effect. Pinobanksine 40 µM caused strong positive inotropism and chronotropism, with no decay at higher concentrations. Hearts exposed to IRI had no significant change with Propolis, while treatment with Pinobanksine showed significant performance improvement, compared with the control. Apitoxin 40 ng/mL produced negative inotropism and chronotropism, with levels changing over time. The rate of arrhythmias increased with doses. Melittin 0.7 mg/mL had slight negative inotropism and chronotropism. Upon IRI, it revealed better recovery compared with both apitoxin 40 ng/mL, and control. Additionally, Melittin at 0.7 mg/mL demonstrated significant cardioprotective effects with no arrhythmias. Results: paired samples T-test, mean+/-S.E.M 25.

Conclusion: These results suggest that certain components of Propolis and Apitoxin have the potential to mitigate oxidative stress IRI in the heart. The biphasic effects may be due to different compounds with distinct affinities in the mixtures.

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P-17

Allyl isothiocianate triggers Ca^{2+} signaling and nitric oxide release via ROS production in human cerebrovascular endothelial cells

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Introduction: An increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) stimulates the production of nitric oxide (NO) in endothelial cells. Allyl isothiocyanate (AITC), a major active constituent of cruciferous vegetables, increases cerebral blood flow, but it is still unknown whether it stimulates NO release in human brain endothelial cells.

Objective: To investigate whether and how AITC induces a Ca^{2+}-dependent NO release in the human cerebrovascular endothelial cell line, hCMEC/D3.

Methods: $[Ca^{2+}]_i$, NO production, and reactive oxygen species (ROS) production were measured by microfluorimetric techniques by loading the hCMEC/D3 with Fura-2/AM, DAF-FM, and H_2DCF-DA, respectively. Transient receptor potential ankyrin 1 (TRPA1) expression was evaluated by immunoblotting. Values are expressed as mean ± S.E.M, n= number of cells analyzed.

Results: In hCMEC/D3, AITC evoked: 1) NO release (latency: 299.3 ± 10.8 s, n= 134); Ca^{2+} signal (latency: 125.2 ± 5.2 s, n= 105; and 3) ROS production (latency: 20.7 ± 1.8 s, n= 72). The Ca^{2+} response to AITC was shaped by both intracellular and extracellular Ca^{2+} sources, was insensitive to the pharmacological blockade of TRPA1, was triggered by cytosolic ROS production, and was supported by store-operated Ca^{2+} entry. Conversely, the Ca^{2+} response to AITC did not require Ca^{2+} mobilization from the endoplasmic reticulum, lysosomes, or mitochondria. The AITC-evoked NO release was driven by ROS generation and required ROS-dependent inhibition of plasma membrane Ca^{2+}-ATPase (PMCA) activity.

Conclusion: This study demonstrates that AITC stimulates NO release from hCMEC/D3 cells through an increase in $[Ca^{2+}]_i$ that is driven by ROS-dependent inhibition of PMCA activity.

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P-18

Effects of diabetes on melanin-concentrating hormone (MCH) peripheral system in a murine model

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Introduction: Diabetes mellitus induces vascular wall remodelling. MCH is a neuropeptide with a crucial role in energy balance affecting glucose metabolism. MCH activates MCHR-1 receptors present in primary cilia from neurons and other cellular phenotypes.

Objective: We study if MCHergic system could be modified in diabetic mice and if it could be associated with changes in primary cilia density in their aortas, comparing with control ones.

Methods: To induce diabetes, C57BL6 adult male mice received daily intraperitoneal injections of streptozotocin (STZ, 60mg/kg, n=18, five consecutive days), whereas control group received vehicle (citrate buffer, n=15). Experimental protocol was approved by the local ethics board (N° 070153-000440-19). Blood glucose and body weight were measured along four weeks. After that, plasma and aorta artery were obtained. Plasmatic MCH levels were measured by ELISA. Primary cilia distribution was determined by immunofluorescence with anti-acetylated tubuline antibodies in *en face* aorta preparations. T-test was used for statistical analysis, and all values are mean±SEM.

Results: STZ increased significantly glucose levels from week 1 to 4 (10.3±1.7 mM vs 15.6±4 mM, $p<0.0001$) and lose body weight at week 4 (26.83±2.8 gr vs 25.07±1.8 gr). Plasmatic MCH levels had an increase tendency in diabetic mice (3157±709 pg/mL vs 2670±577 pg/mL; n=13 and 11). Aorta cilia density had an increased tendency in the aortic arch of diabetic mice (5.7±2.8 vs 3.9±0.7 cilia/5000 μm^2; n=3) and a decreased tendency in abdominal aorta (2.3±0.7 vs 5.1±1.5; n=3), whereas was unmodified in thoracic region (2.0±0.6 vs2.5±0.6; n=3).

Conclusion: Our results suggested that MCH peripheral system could be involved in diabetes induced-vascular changes.

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P-19

The consequence of antagonistic regulation of the mitochondrial protein MUL1 by norepinephrine and 17 beta-estradiol on cardiomyocyte hypertrophy

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Introduction: MUL1, an E3 ubiquitin ligase anchored to the outer mitochondrial membrane highly expressed in the heart, is involved in mitochondrial dynamics and function. Moreover, cardiovascular risk is higher in men than in premenopausal women of the same age, showing that estrogen (E2) has a cardioprotective effect.

Objectives: 1) To study in vitro the effects of E2 on norepinephrine (NE)-induced cardiomyocyte hypertrophy (CH) and whether E2 regulates MUL1 protein levels. 2) To evaluate changes in cardiac function and structure in a mouse model with cardiomyocyte-specific overexpression of MUL1.

Methods and Results: Cultured neonatal rat cardiomyocytes (NRCM)(Protocol CICUA-20351-CYQ-UCH) were preincubated with or without E2 (100 nM) before NE treatment for 48 h. NE increased cell area, atrial natriuretic peptide (ANP) mRNA and protein, MUL1, mitochondrial network fragmentation, and decreased ATP levels significantly. E2 prevented all these effects. Knockdown of MUL1 in NRCM treated with NE reduces ANP mRNA and protein levels significantly. In contrast, overexpression of MUL1 increases cell area. Data are shown as mean ± SEM (n=4); ANOVA. Additionally, transgenic mice overexpressing MUL1 males (n=10) and controls (n=5) at 35 weeks of age were evaluated. The glucose tolerance curve, blood pressure, and diastolic pressure did not show significant changes between the groups. However, significant changes were observed in hypertrophy (heart weight/tibia length) and diastolic function assessed by echocardiography.

Conclusion: E2 prevented CH, mitochondrial fragmentation, and MUL1 protein levels. Accordingly, cardiomyocyte-specific overexpression of MUL1 in mice affects myocardial function, suggesting a critical role of MUL1 in the cardiomyocyte.

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P-20

Vasomotor activity of the extract of Weinmannia trichosperma Cav. (Tineo) in rat aorta

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Introduction: *Weinmannia trichosperma* Cav. (Tineo) is an endemic tree commonly employed in Mapuche culture as a medicinal plant in the management of various metabolic disorders. The bark is rich in phenolic compounds that are known for their bioactivity, mostly their antioxidant capacity. There are no reports on the vascular myogenic activity of the ethanolic extract.

Objetive: The major aim of this study is to evaluate the effects of *W. trichosperma* ethanolic extract on vascular reactivity using aortic rings.
Methods: To evaluate the relaxation effect of the extracts, aortic rings (2-3 mm) were mounted in an organ bath with Krebs-Ringer bicarbonate solution (KRB) pH 7.4, 37° C, 95% O_2 and 5% CO_2. The intact or endothelium-denuded aortic rings were precontracted with 10^{-6} M phenylephrine (PE), in the presence or absence of *W. trichosperma* extract (0.1 to 100 µg/mL). Two-way ANOVA and *post-hoc* Bonferroni test was used for statistical analysis ($p < 0.05$), following approval by the Ethics Committee of Universidad de Antofagasta (CEIC-275/20).
Results: *W. trichosperma* extract (10 µg/ml) induced vascular relaxation in the presence of endothelium (119±5 % control vs 13±2 % denuded). The preincubation with extract 10 µg/ml significantly ($p<0.05$) decreased the contractile response to phenylephrine 10^{-5} M (EC_{50} 4.4 x 10^{-8} M control vs 1.84 x10^{-8} M Tineo) but did not reduce the contractile response to KCl.
Conclusion: Preincubation with the extract significantly decreased the contractile response to PE. *W. trichosperma showed* a potent endothelium-dependent vascular relaxation effect, via NO mechanisms.
Financing: Financial support was provided by FONDECYT 1180059 and 1220075 to M.J.S., FONDECYT 1200610 to J.P., the Network for Extreme Environments Research project to F.C. and A.P. (NEXER, Project ANT1756, Universidad de Antofagasta, Chile). R.B. received funding from ANID PFCHA/ beca doctorado nacional/2019-21191978.

P-21

Human placental microvascular endothelium proliferation and intracellular pH are modulated by high extracellular D-glucose and activator protein 1 in mothers with pre-pregnancy normal weight

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Introduction: Gestational diabetes mellitus (GDM) alters placental microvasculature angiogenesis which is mediated by activator protein 1 (AP1).
Objective: To determine the AP-1 and intracellular pH (pHi) role in human placental microvascular endothelium proliferation exposed to high extracellular D-glucose (HG).
Methods: Human placental microvascular endothelial cells (hPMECs) were from women with normal pregnancies and with pre-pregnancy normal weight(N*nw*), overweight(N*ow*), or obesity(N*ob*) (n=3-10) (UMCG, U Groningen, NL, with patient consent, Ethics N°RR10959). Cell migration was determined by wound healing assay (24 h), and proliferation by BrdU incorporation (24h). The pHi and recovery rate (*dpHi/dt*) were measured by the acid pulse assay in BCECF-AM(12 µmol/L,10 min)-preloaded cells in the absence or presence of SR11302 (AP1 inhibitor, 10 µmol/L,24h).
Results: Proliferation was reduced ($P<0.05$, unpaired *t*-test, mean±SEM) by HG (23±2% and 33±7% for 10 and 25 mmol/L, respectively) in Nnw. The wound closure was lower in hPMECs incubated with 25 mmol/L (61 and 45%,n=2) versus 5 mmol/L (79±3%,n=3) D-glucose. Basal pHi in Nnw (7.36±0.07 pHi) is reduced ($P<0.03$) in 0.15 pHi units by SR. However, SR did not alter the basal pHi in Now and Nob. Only the dpHi/dt in Nnw (0.0026±0.0004 pHi units/min) was reduced ($P<0.05$) by SR. Cells from GDMnwe showed basal pHi 7.87 and 7.46 in the absence and presence of SR. Also, the dpHi/dt was 0.004 pHi units/min in

the absence and potentiated (2.4-fold) by SR in GDMnw.

Conclusion: HG inhibits the proliferation of hPMECs. Also, the AP1 activity seems to maintain the basal pHi and pHi recovery in cells only from Nnw.

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P-22

Long-term modulation of vascular reactivity by testosterone and aldosterone: An investigation in orchiectomized wistar rats treated with spironolactone

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Introduction: Testosterone, has been shown to exhibit endothelium-dependent vasodilation in acute scenarios.

Objective: We test the hypothesis that testosterone plays a major role in the long-term modulation of vascular reactivity by an aldosterone-dependent pathway.

Methods: 12-week-old Wistar rats were segregated into Control (SHAM, N=8) and Orchiectomy (OQT, N=9) groups and treated for 3 months with spironolactone (SPI) (SHAM+SPI, N=10 and OQT+SPI, N=9, 80 mg/kg, gavage), aldosterone receptor antagonist. (CEUA-UFES 17/2020). Vascular reactivity was examined in isolated thoracic aorta rings during concentration-response curves to phenylephrine (Phe) (10^{-11} to 10^{-3} M) in the presence and absence of L-NAME (LN) (NOS inhibitor, 100 µM), and with endothelium-denuded rings (E-). Results were expressed as mean±SEM.

Results: After 3 months, the groups OQT showed less body weight gain compared to the SHAM. This discrepancy was abolished with SPI treatment. (SHAM:231±11; OQT:158.4±13*; OQT+SPI:180.5±20.60*; SHAM+SPI:215.3±26.1 g *p<0.05). There was no difference in the maximum response (Rmax) to Phe between the SHAM and OQT groups. However, OQT+SPI group demonstrated a decreased Rmax to Phe compared to the SHAM (SHAM:109.4±9.5%; OQT:120.5±10%; SHAM+SPI: 120.4±7.56 % N=10 vs OQT+SPI: 93.3±10.2 % N=10; *p<0.05). The reactivity to Phe increased in the presence of LN and in E-, with no difference between the groups.

Conclusion: Our data suggest that testosterone has a role in angiotensin II-mediated NO production, since blocking NO production with LN resulted in a decrease in Rmax in the OQT group. It highlights the potential of testosterone in the contractile response mediated by the angiotensin AT1 receptor.

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P-23

PM2.5 exacerbates foam cells formation, by leading to inflammation and impairing heat shock response

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Introduction: Fine particulate matter ($PM_{2.5}$), an air pollutant, enhances the susceptibility to atherosclerosis. The phagocytosis of oxLDL by macrophages is a fundamental trigger for foam cell generation. In this context, 70 kDa-heat shock proteins (HSP70) are powerful anti-senescence and anti-inflammatory chaperones for cardiovascular protection.

Objective: We investigated if the atherogenic effect of $PM_{2.5}$ could be related to an impairment in HSP and inflammatory signaling in macrophages.

Methods: $PM_{2.5}$ retained in filters was partially extracted in PBS and centrifuged at 1000*x*g. This solution (1 g filter/125 mL PBS) was diluted in DMEM 10% FBS ten times. We exposed the RAW264.7 macrophage cell line to $PM_{2.5}$ for 48 h and used PBS as Control. Triglycerides intracellular accumulation by AdipoRed staining; HSP70 by immunocytochemistry in a flow cytometer; IL-6 and IL-10 by ELISA. Each experiment was performed three times. Data were expressed in mean±SEM and compared through t-test, considering P<0.05.

Results: We exposed macrophages to $PM_{2.5}$ for 48 h and added native LDL (50 µg/mL) at the last 24 h. As expected, LDL induced triglycerides accumulation, which was exacerbated by the pollutant (Ctrl: 1.00 ± 0.02; LDL: 1.27±0.06; $PM_{2.5}$: 1.26±0.11; LDL+$PM_{2.5}$: 1.39±0.06*; Two-way

ANOVA: LDL: P=0.009; $PM_{2.5}$: P=0.01; Interaction: P=0.34). $PM_{2.5}$ also increased HSP70 average (Ctrl: 1.00±0.04; $PM_{2.5}$: 1.52±0.14; P=0.005), and IL-6 levels (Ctrl: 1.00±0.23; $PM_{2.5}$: 4.33±0.90; P=0.002), whilst decreased IL-10 release (Ctrl: 1.09±0.12; $PM_{2.5}$: 0.68±0.07; P=0.01).

Conclusion: $PM_{2.5}$ exacerbates foam cell formation by leading to inflammation and impairing heat shock response.

Financing: Fellowship CAPES-PROEX.

P-24 ★*selected for oral communication*

Post-traumatic stress disorder (PTSD) induces a greater negative effect on the cardiovascular system in females compared to males

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Introduction: Post-traumatic stress disorder (PTSD) is a risk factor for cardiovascular disease. Rodent models demonstrate that females are resilient to the effects of traumatic stress however, whether this translates to the heart is unknown. We hypothesized that PTSD impairs cardiac function in males more than females who demonstrate PTSD-like behavioral phenotypes.

Methods: To induce experimental PTSD, male and female C57BL/6 mice (n=9-14/sex, 4.6±0.5 months old) were exposed to 5 foot-shocks (IFS; 1.0 mA, 1 sec duration) in 6 min (Protocol 685 approved by RJH IACUC). Control mice (n=4/sex) were placed in the chambers with no foot shocks. Behavioral testing was performed to characterize mice as PTSD-like mice (n=5 for males; n=5 for females). Doppler echocardiography was collected at serial time points, 0, 2, 4, and 8-weeks post-IFS. Two-way ANOVA was performed.

Results: Female PTSD-like mice had a more drastic impairment in diastolic dysfunction 8 weeks post-IFS compared to male PTSD-like mice as demonstrated by a more drastic decrease in aortic ejection time ($p<0.01$), ejection fraction ($p<0.01$), and an increase in isovolumetric relaxation time (IVRT; $p<0.001$). Interestingly, females began to show impairment earlier at 4-weeks post-IFS than their male counterparts as demonstrated by an increase in IVRT ($p<0.001$). To determine a potential mechanism, we evaluated matrix metalloproteinase (MMP) activity in cardiac tissue at 4-weeks post-IFS which demonstrated that male but not female PTSD-like mice had increased activity compared to controls.

Conclusion: Our data demonstrates that in a mouse model of PTSD, female mice show an earlier cardiac phenotype compared to males.

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P-25

Lateral hypothalamic astrocytes play an excitatory role in the hypercapnic chemoreflex in a light/dark cycle-dependent manner

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Introduction: Astrocytes have been shown to play a fundamental role in central CO_2 chemoreception, either by interacting with neighboring chemosensitive neurons or through their inherent ability to sense CO_2/H^+. Nevertheless, the possible contribution of LH/PFA astrocytes in the control of hypercapnic chemoreflex remained unexplored.

Objective: To investigate if latero-hypothalamic astrocytes contribute to the hypercapnic ventilatory response control and if this possible role is influenced by the sleep-wake and light-dark cycles.

Methods: We pharmacologically manipulated the activity of astrocytes in the LH/PFA of non-anesthetized male Wistar rats, through microinjection of fluorocitrate (Fct), which selectively depolarizes astrocytes. The respiratory parameters were evaluated by whole-body

plethysmography, together with EEG/EMG and body temperature, under 7% CO_2 hypercapnia, during wakefulness and NREM sleep, in the light and dark phases. All procedures were done with the approval of the local Animal Care and Use Committee (CEUA# 597). Values are reported as means ± SD. Data were analyzed by two-way ANOVA, followed by Bonferroni's post-hoc test. The significance level was set at $P < 0.05$.

Results: Fct did not alter the hypercapnic chemoreflex during the light phase but increased the hypercapnic ventilatory response of the rats during the dark phase in both states of consciousness, compared with the control group (2817 ± 451; n = 8 versus 2209 ± 253 mL kg-1 min-1; n = 6; P = 0.009).

Conclusion: Our results demonstrate that astrocytes in the LH/PFA play an excitatory role in the control of CO_2 ventilatory response in a light-dark cycle-dependent manner.

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P-26

NOX2 and NLRP3 inflammasome mediates the cardiac protection by remote ischemic preconditioning

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Introduction: Short episodes of ischemia-reperfusión (IR) performed directly in the heart (classical ischemic preconditioning, IPC) or in a distant tissue (remote ischemic preconditioning, RIPC) before a prolonged ischemic episode, reduce ischemia-reperfusion damage. It is unknown whether IPC and RIPC share common mechanisms of protection. Animals K.O. for NOX2, a superoxide-producing enzyme, or K.O. for NLRP3, a protein component of the inflammasome, are not protected by IPC.

Objective: To investigate if NOX2 or NLRP3 inflammasome are involved in the protection induced by RIPC.

Methods: Hearts were obtained from control rats or after 4 x 5 min periods of IR in the limb (RIPC). NOX2 subunit content and activity and NLRP3 inflammasome subunits were measured in heart homogenates or membrane-enriched fractions. Infarct size after 30 min of ischemia followed by 60 min reperfusion was measured with or without NOX2 inhibitor (apocynin) or NLRP3 inhibitor (Bay117082). Data was analyzed by one-way ANOVA. The Institutional Ethics Review Committee, protocol number CBA 22547-MED-UCH approved this work.

Results: RIPC increased NOX2 activity as indicated by the association of p47 to the membrane and by the increased oxidation rate of NADPH. NLRP3, procaspase-1, and caspase-1, components of the NLRP3 inflammasome, were all increased in the hearts of RIPC rats. RIPC decreased the infarct size after IR from 39,4 ± 8,1(N=15) to 8,9 ± 3,2 (N=7) ($p<0.0001$). This protective effect was lost in the presence of both inhibitors.

Conclusions: NOX2 and NLRP3 inflammasome are involved in the protection induced by RIPC in the heart.

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P-27

Exosomes from patients with ischemic stroke differ in number, size, composition, and adverse effect over the blood-brain barrier, depending on the clinical severity

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Introduction: Circulating exosomes from patients with ischemic stroke (i-stroke) may disrupt the blood-brain barrier (BBB).

Objective: To investigate whether exosomes from patients with mild and severe i-stroke exhibit a differential phenotype, content, and effect over the BBB.

Methods: I-stroke patients, acute phase (24-48 h, n=18) and controls (n=6) were matched by age, gender, and blood pressure. I-Stroke was classified

in severe and mild clinical features. Serum exosomes were characterized in size, concentration, and protein markers (CD63, Alix, CD81, TSG101, and HSP70). Exosomes content of VEGF, PIGF, nitrotyrosine, CLDN-5, IL-6, and TNF-α was estimated. Exosomes uptake, cell viability, electrical resistance (TEER) and Dextran-70kDa permeability were analyzed in hCMEC/D3. Kruskal-Wallis and Dunn's multiple comparison tests were used. Data presented as mean±SD. Ethical approval code CEC-HCHM#15-177.

Results: Exosomes isolation from serum was achieved. Patients with i-stroke have lower concentration of exosomes than controls (p=0.005). Patients with mild i-stroke have the smallest exosomes. Exosomes from mild i-stroke have higher content of IL-6 and TNF-α; while exosomes from severe i-stoke has higher VEGF but lower PlGF than controls (p<0.05). Exosomes content of nitrotyrosine and CLDN5 levels were higher in severe and mild i-stroke than controls (p<0.05). Exosomes (mild and severe) were uptake by hCMEC/D3, which didn't impair cell viability. However, exosomes from severe i-stroke induce higher drop in the TEER than controls.

Conclusions: Exosomes from patients with i-stroke differ in number, size, and composition of angiogenic and inflammatory molecules depending on the severity. This imbalance could explain higher compromise of the BBB in severe i-stroke.

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P-28

Mitoquinone treatment prevents cardiomyocyte hypertrophy, changes in contractility, and mitochondrial oxidative stress induced by myocardial infarction in rats

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Introduction: Myocardial infarction (MI) induces contractility dysfunction dependent on oxidative stress.

Objective: We investigated the effects of a 7-day MitoQ treatment, a specific mitochondrial antioxidant, on cardiomyocyte contractility, Ca^{2+} transient, and mitochondrial oxidative stress 7 days after MI in rats.

Methods: Rats were divided into Sham (S, n=9), Sham MitoQ (SM, n=8), MI (n=6), and MI MitoQ (n=7) groups (Ethical Committee 16/2021). During one week rats in the MitoQ groups received MitoQ diluted in drinking water (100 µM). Cardiomyocytes (CMs) were isolated and experiments were conducted to evaluate CMs morphometry, contractility, Ca^{2+} transient (Fluo-4/AM), and mitochondrial reactive oxygen species production (MitoSOX Red). Statistical analysis: Two-way analysis of variance (ANOVA) and Tukey's post-hoc test. Results were presented as mean±SEM.

Results: Morphometric analyses showed that MitoQ treatment prevented the increase in cellular area and cellular length of CMs. Contractility analysis revealed increased parameters in the MI, which were mitigated by MitoQ, including CMs shortening (MI:815±30; MIM: 53.6±30* µm^2, *P<0.05), maximum contraction velocity (MI:87.76±3; MIM:60.2±3* µm/s, *P<0.05), and maximum relaxation velocity (MI:67±2.4; MIM:43.6±2,55* µm/s, *P<0.05). MitoQ treatment prevented the increase of the Ca^{2+} transient amplitude induced by MI (MI:3.15±0.07; MIM:2.3±0.05*, *P<0.05). The evaluation of mitochondrial anion superoxide production revealed an increase in MI, compared to Sham group, which was significantly reduced by MitoQ treatment (MI:102±7.40; MIM:40.65 ± 9.69*, *P<0.01).

Conclusion: In isolated CMs, MitoQ treatment prevented the hypertrophy induced by MI after 7-days. Additionally, it attenuated the increase in CMs contractility which may be linked to altered Ca^{2+} handling and mitochondrial oxidative stress.

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P-29

Distinguishing right ventricular contractility post-myocardial infarction in rats with and without heart failure signs

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Introduction and Objective: In this study, we investigated the right ventricle (RV) contractility during the early phase (7 days) following myocardial infarction (MI) in rats with equivalent scar sizes (SS), distinguishing between those exhibiting signs of heart failure (HF) and those without HF.

Methods: Male Wistar rats were categorized into: control (SHAM; n=10), infarct (INF; n=10), and infarct with HF (HF; n=6). Hemodynamic assessments were conducted seven days post-MI, and Langendorff perfusion was utilized for isolated heart analysis. RV contractility was evaluated through RV pressures and the first temporal derivative of pressure (dP/dt) in the presence of isoproterenol (10^{-5} M) and Ca^{2+} 0.62 to 3.5 mM. Statistical analysis involved mean ± SEM, Two-way ANOVA, and Bonferroni post-hoc test, *p<0.05 (Local Ethical Comitte:03/2007).

Results: The INF and HF groups exhibited identical SS (INF=31.6±1.6; HF=30.8±0.8%). RV systolic pressure (RVSP,mmHg) and RV end-diastolic pressure (RVEDP,mmHg) were elevated in the HF group while remaining unchanged in the INF group (RVSP:SHAM=29±2.2; INF=28±2.2; HF=40±2.3*#mmHg; RVEDP: SHAM=1.1±0.2; INF=1.33±0.3; HF=2.2±1.2*#mmHg; dP/dt+ RV:SHAM=971±191; IC=1915±210*mmHg/s). Langendorff-perfused hearts from the HF group exhibited reduced RV isovolumic systolic pressure (RVISP,mmHg), +dP/dt, and -dP/dt in response to increased Ca2+ concentration and isoproterenol, while these parameters remained preserved in the INF group.

Conclusion: Our study demonstrates that following MI, rats with equivalent scar sizes may either develop HF or remain HF-free, and the reduction in RV contractility is specifically observed in infarcted animals exhibiting signs of HF. These findings shed light on the complex dynamics of RV function in the context of post-MI outcomes.

Financing: FAPES, UFES, CNPq.

P-30

Role of BIN1 in the differentiation of cardiac fibroblasts into myofibroblasts

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Introduction: Differentiation of cardiac fibroblasts (CF) to myofibroblasts (MyF) is involved in fibrosis, remodeling, and cardiac dysfunction. BIN1 is expressed and is crucial for cardiac function, however, its function in CF is not known. c-Myc inhibit BIN1 expression in cancer cells.

Objective: To determine whether BIN1 is related to the differentiation of CF to MyF.

Methods: Primary cultures of CF isolated from neonatal (1-3 days old) Sprague Dawley rats were used. Differentiation of CF into MyF was induced with TGF-β1 (10 µM, 72 h). α-SMA, BIN1, and c-Myc proteins and mRNA levels were measured by Western blot (Wb) and qRT-PCR, respectively. BIN1 and c-Myc were silenced with a specific siRNA and c-Myc was overexpressed with adenoviral vector. Student's unpaired t test or one-way ANOVA and Tukey's post-test were used for statistical analysis, and data were expressed as the mean ± SEM (n=4-6). FMUCH#0997.

Results: α-SMA, a marker of CF differentiation, as well as BIN1 protein and mRNA were increased with TGF-β1, while c-Myc expression decreased.

BIN1 knockdown or c-Myc overexpression prevents increased of α-SMA levels. Moreover, c-Myc knockdown avoid BIN1 increased induced by TGF-β1.

Conclusion: BIN1 is required for CF differentiation to MyF, at least in part, through c-Myc pathway inhibition.

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P-31

Role of the primary cilium in maintaining the contractile phenotype of vascular smooth muscle cells

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Introduction: Vascular smooth muscle cells (VSMC) regulate blood pressure through their contractile machinery. Under pathological conditions, VSMC loss this contractile phenotype and change to a secretory phenotype, which contributes to the development of vascular pathologies. Mechanical stretch causes dedifferentiation of VSMCs from contractile to secretory phenotype. The primary cilium is a cellular organelle specialized in the transduction of mechanical signals. Loss of primary cilium induces ciliopathy, where there is a predisposition to obesity, renal and vascular diseases, however, the role of this organelle in VSMCs during mechanical stretch its unknown.

Objective: To study the role of the primary cilium in maintaining the contractile phenotype of vascular smooth muscle cells under mechanical stress conditions.

Methods: A7r5 cell line (VSMC) was used. Hypoosmotic solution (HS, 2 y 24 h) was employed to induce mechanical stress. Cilia were disassembled using an siRNA-IFT88. Phenotype was assessed by markers through Western-blot and RT-qPCR (α-SMA, SM22, calponin, Collagen I, Collagen III). Primary cilium was identified by immunocytochemistry. For statistical analysis, t-test or ANOVA was used, followed by Tukey's test. Differences were significant when $p < 0.05$. n=4-5.

Results: Mechanical stress induces a decrease in contractile markers, whereas in the absence of the primary cilium the markers are preserved at basal levels in VSMC.

Conclusions: The primary cilium is crucial in the loss of the contractile phenotype of vascular smooth muscle cells during mechanical stress.

Financing: Fondecyt regular 1230650 (Pedrozo Z) and 1220392 (Chiong M). ACCDiS FONDAP 15130011.

P-32

Insulin regulates MUL1 expression in cultured skeletal and cardiac muscle cells

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Introduction: Insulin regulates mitochondrial dynamics and function by Akt, generating an elongated mitochondrial phenotype and stimulating mitochondrial oxidative metabolism. MUL1, a mitochondrial E3 ligase, ubiquitinates Akt and Mfn2, targeting proteasomal degradation. MUL1 also increases Drp1-induced mitochondrial fragmentation and decreases insulin-induced mitochondrial metabolism. Furthermore, MUL1 expression significantly increases under insulin resistance conditions. Thus, MUL1 can potentially regulate insulin sensitivity. However, the role of MUL1 on insulin signaling has been poorly explored in muscle cells.

Objective: We aimed to evaluate the effect of insulin on MUL1 expression in cultured skeletal and cardiac cells.

Methods: We studied the effect of insulin (10 nM) on MUL1 protein level by Western bot in cultured rat L6 myoblasts, mouse C2C12 myoblasts, and neonatal rat cardiomyocytes (NVRM) under insulin stimulation at different times. All procedures were approved by CICUA Committee (20367-CQYF-UCH). Data were analyzed by ANOVA; values correspond to mean ± SEM of at least three independent experiments.

Results: Insulin increased MUL1 expression levels in L6 myoblasts until 4 h post-stimulation. No changes in MUL1 protein level were observed in insulin-stimulated NRVM. However, the MUL1 protein level decreased in C2C12 myoblasts after 4 h PS with insulin.

Conclusion: Our results suggest that insulin could differentially regulate the MUL1 expression or activity in skeletal muscle cells. Also, insulin could induce mitochondrial fusion and oxidative metabolism by MUL1-dependent NF-κB activation, which increases the Opa1 expression. Further studies are required to test this hypothesis.

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AREA: ENDOCRINE AND METABOLISM

P-33 ★*selected for oral communication*

Human adipose-derived extracellular vesicles (AdEVs) increase proinflammatory markers in renal and endothelial cells: A preliminary study

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Introduction: In obesity, the white adipose tissue (WAT) secretome including adipokines and adipose-derived extracellular vesicles (AdEVs) would play a key role in obesity progression and comorbidities.

Objective: To characterize and evaluate the AdEVs effect in renal cells (RC) and endothelial cells (EC) cultures.

Methods: AdEVs from human SW872 adipocytes cultured in T875 flasks (n=3, each condition) were isolated by ultracentrifugation and characterized by NTA, TEM and w-blot. AdEVs (1x10^3), then were added to RC (human collecting duct, HCD) and EC (EaHy926) cells for 24-hours. Expression of adipogenic pathway, inflammation (IL-6 and IL-1B) and eNOS genes were evaluated in cells and AdEVs by RT-qPCR. Statistic was performed by Mann-Whitney test and expressed as mean+-SE.

Results: Isolated AdEVs from SW872 have a donut shape morphology, size (50-150 nm) and EVs markers CD9 and Tsg101. EVs showed similar relative expression of adiponectin, PPARγ and FASN than their parental cells. Either RC or EC treated with AdEVs express higher levels of IL-6 (RC 2.3+-0.8 vs 1.0+-0.2; EC 2.1+-0.4 vs. 1.0+-0.2; $p<0.05$) and IL-1B (RC 2.8+-0.5 vs 1.0+-0.2; EC 2.2+-0.8 vs 0.8+-0.2, $p<0.05$) that untreated cells. eNOS was decreased in EC treated with AdEVs (0.7+-0.2 vs 1.0+-0.2; $p<0.05$).

Conclusion: AdEVs have similar relative gene expression as their parental cell. Treatment of both RC and EC with AdEVs showed an increase of IL-6 and IL-1B, and decreased eNOS (in EC). These results suggest novel roles of AdEVs, and its cargo, in inflammation (RC & EC) and endothelial dysfunction (EC), both involved in comorbidities associated to obesity.

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P-34

Irisin levels can help reduce methylation age even in adults who struggled with obesity from a young age

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Introduction: Exerkines are signaling molecules released during exercise, helping improve health, longevity, and resilience. Conversely, obesity increases the risk of premature aging by elevating methylation age (DNAmAge). This can compromise longevity and overall health.

Objective: To investigate whether certain exerkines can reduce DNAmAge in adults long exposed to obesity.

Methods: Multiple-events case-control study in a Chilean birth cohort. In 174 participants (49% women) BMI was estimated periodically since birth; three trajectories were traced (cubic polynomials): always healthy BMI (TG1), adolescent obesity (TG2), and childhood obesity (TG3). At 29y, we determined apelin, irisin, myostatin, osteocrin, oncostatin, and osteonectin (Luminex), and DNAm age. Multilevel modeling was used to conduct analyses. Certified IRB granted ethical permission (12-2021; Nº21–037).
Results: In the sample (Mean, ±: 28.9y, 0.6y), 40% fell into TG3 and 24% into TG2; no sex differences. In TG1, DNAmAge was significantly associated with lower apelin (B:-2.74; P=.04), oncostatin (B:-3.91; P=.005), irisin (B:-2.64; P=.01), and osteonectin (B:-2.22; P<.05). In TG2 and TG3, although the regression coefficients were negative, they were not statistically significant, except for irisin (TG2, B:-2.13; P<.05 | TG3, B:-2.03; P<.05). After controlling for sex, irisin effect on DNAmAge remained significant in all trajectories. Myostatin and osteocrin were unrelated to DNAmAge in the sample.
Conclusion: While several exerkines had the potential to lower DNAmAge, only irisin could effectively reduce it regardless of weight status and the duration of obesity in both males and females. This underscores the importance of irisin as potential therapeutic option to combat aging and related diseases.
Financing: ANID funded grants: ACT210006, FONDECYT1210283. Other grants: NIH-02RHL088530, MAPFRE21-01.

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GLP1 receptor activation improves glucose tolerance in ob/ob mice by leptin independent mechanisms

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Introduction: Glucagon-like peptide 1 (GLP1) and leptin regulate blood glucose by increasing insulin sensitivity at peripheral and central levels. Whether the effects of GLP1 on glycemia require the activity of leptin is unknown.
Objective: To determine whether leptin activity is necessary for GLP1 regulation of glucose homeostasis.
Methods: Male and female obese leptin-deficient (*ob/ob*) (n=5) and wildtype (WT) (n=7) mice were injected intraperitoneally with the following combinations, separated by 15 min: vehicle/vehicle, leptin/vehicle, vehicle/Exendin 4 (EX4, GLP1 receptor agonist), leptin/Exendin 9 (EX9, GLP1 receptor antagonist) (leptin: 1 mg/kg, Ex4: 10 mg/kg, Ex9: 100 mg/kg). An intraperitoneal glucose tolerance test (GTT) was performed 15 min after the second IP injection. The area under the curve (AUC) was analyzed during the GTT using a two-way ANOVA with genotype and treatment as independent variables (mean ± S.E.M). All protocols and treatments were approved by bioethics committee of Pontificia Universidad Católica de Chile (210421002).
Results: As expected *ob/ob* mice had glucose intolerance compared to WT mice (larger AUC). Leptin sole injection or in combination with Ex9 did not changed glucose tolerance in *ob/ob* or WT mice, however, a single injection of Ex4 improved glucose tolerance by 40% in *ob/ob* and WT mice compared to animals inject with vehicle/vehicle ($p < 0.05$).
Conclusions: The pharmacological activation of GLP1 receptor was sufficient to improve glucose tolerance in WT and leptin deficient *ob/ob* mice while its blocking did not affect glucose tolerance in these animals. These results suggest that GLP1 regulates glucose homeostasis by leptin-independent mechanisms.
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Acknowledgments: ANID ACT210039

P-36 ★*selected for oral communication*

Sex-modulated transcriptomic changes in the interaction between leptin, GLP-1, and glucocorticoid receptors in the postprandial response to chronic high-fat diet

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Introduction: The hypothalamus is a key brain area regulating energy balance by integrating neuroendocrine metabolism. High-fat-diet (HFD) disrupts neuroendocrine control, including sensing of anorexic signals such as leptin, GLP1, and glucocorticoids. However, males and females respond differently to HFD, but the transcriptional mechanism underlying this sexual dimorphism remains unclear.

Objective: To determine the hypothalamic transcriptomic changes underlying the sexual dimorphic response to chronic HFD-feeding and their association with leptin, GLP1, and glucocorticoids pathways.

Methods: Male and female C57BL6/J mice were fed an HFD or low-fat-diet (LFD) for 12-weeks. Hypothalamic gene expression changes were determined by RNA-Seq from preprandial samples or after 3h of food-access (n=4 per sex, diet, and prandial state) using Log2FC≥1 or ≤1 and p_adj<0.05 using Benjamini-Hochberg correction. The interaction between differentially expressed genes by prandial state exclusively regulated in each sex with leptin, GLP1, and glucocorticoids pathways was compared. The protocol was approved by bioethics committee of PUC (210421002).

Results: HFD feeding induced body weight gain solely in males. The RNA expression pattern was compared and there were 359 differentially expressed genes between males and females by prandial state in HFD vs 633 in LFD. Protein-protein interaction network analyses in males revealed interaction between GLP1R and GNG7, a gene related to addiction disorders. Glucocorticoid receptor interacts with circadian rhythm-associated genes. LEPR did not interact with male:female differentially expressed genes.

Conclusions: The results showed a differential transcriptional response when comparing pre and postprandial state, with sex-specific and HFD-specific changes, identifying candidate genes for studying hypothalamic neuroendocrine integration.

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Human equilibrative nucleoside transporters 2 protein abundance is increased in women with pre-pregnancy overweight that develop gestational diabetes mellitus

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Introduction: Reduced adenosine uptake and increased endothelial nitric oxide synthase (eNOS) activity is reported in foetoplacental endothelium from gestational diabetes mellitus (GDM).

Objective: To characterize the effect of pre-pregnancy weight in GDM on the foetoplacental endothelial function.

Methods: Extracted human umbilical vein endothelial cells (HUVECs) were from women with normal pregnancies and pre-pregnancy normal weight (N*nw,* n=3-4), overweight (N*ow,* n=1-2) or obese (N*ob*, n=3-4) and GDM*nw*, GDM*ow* or GDM*ob* (n=1-2) (Clinical Hospital UC CHRISTUS, with patient consent, Ethics #012793). Cells fixed for immunofluorescence were assayed for human equilibrative nucleoside transporter 2 (hENT-2) expression, total endothelial nitric oxide synthase (eNOS), Ser^{1177} phosphorylated-eNOS (P-eNOS), and human Na^+/H^+ exchanger 1 (hNHE1). Mann-

Whitney U Test was performed on N*nw* and N*ob*.
Results: Total eNOS protein abundance was higher (3.9 ± 0.7 fold, *P*<0.05, mean ± SEM) in cells from N*ob* compared with N*nw*. In GDM*ow* the P-eNOS/eNOS ratio decreased (56 ± 6 %) but total eNOS increased (2± 0.2 fold) compared with N*ow* cells. hENT2 protein abundance was higher in GDM*nw* (2.03 ± 0.2 fold) and GDM*ow* (1.6 ± 0.5 fold) compared to N*nw* and N*ow*, respectively. However, hENT2 protein abundance decreased (45 ± 20%) in GDM*ob* compared to N*ob*. hNHE1 was lower (37 ± 4%) in GDM*nw* compared to N*nw*, but it was higher (1.43 ± 0.2 fold) in GDM*ob* compared to N*ob*.
Conclusion: Gestational diabesity is a metabolic condition that associates with differential regulation of the expression of nucleoside and H+ transporters compared with cells from N*nw*, N*ow or* N*ob*.
Financing: VRI+DIDEMUC-PUC, Univ. de Talca Chile. LS Sao Paulo Research Foundation-FAPESP [Grant-FAPESP 2016⁄01743–5], Brazil. MC and GF hold fellowships from the ATTP-UMCG, and De Cock-Hadders MC(n°2023-51), GF(n°2022-12 / 2023-29). GF, MC, and PV hold PhD fellowships from ANID [21221950, 21222280, and 21221870]. Univ. de Antofagasta

P-38
Restitution of leptin reduces overall consumption of sucrose without changes of elasticity in a behavioral-economic model.
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Introduction: The deficit in leptin, a hormone produced by the white adipose tissue, increases food intake and is associated with higher motivation for food, including tasty foods such as sucrose. However, whether leptin alters the elasticity of demand and palatability of tasty foods is unknown.
Objective: To determine if leptin reduced the demand elasticity and palatability of sucrose.
Methods: Leptin-deficient mice (*ob/ob*) were tested in a demand curve for sucrose before and after implantation of a pump for leptin (n=3, dose=0.25 ug/hr) or saline (n=3) delivery for 14 days in *ob/ob* mice. WT mice were tested before and after a sham surgery (n=4). Number of sucrose rewards obtained, elasticity, and palatability for sucrose (mean±SEM) were analyzed with ANOVA or Student's t-test. Experiments were approved by the IACUC at UC (230426002).
Results: Before surgery, palatability for sucrose was 62.6±8.2% larger in *ob/ob* compared to WT mice (P<0.05), while elasticity was not different between genotypes (P=0.18). Compared to before surgery, the number of sucrose rewards obtained was reduced by 16.5±7.5% in *ob/ob* mice administered leptin, while it increased by 15.8±7.3% in *ob/ob* administered saline and by 62.9±9.9% in WT mice (P<0.05). While elasticity and sucrose palatability increased in WT mice after sham surgery (P<0.05), leptin or saline did not alter these parameters in *ob/ob* mice.
Conclusion: Leptin deficiency is associated with increased palatability but not larger demand elasticity for sucrose. Leptin restoration reduced sucrose intake in a demand test, but did not change demand parameters for sucrose.
Financing: ANID ACT210039
Acknowledgments: Funding provided by ANID ACT210039

P-39 ★*selected for oral communication*
Andro-mediated modulation of glucose metabolism in visceral adipose tissue in Alzheimer's disease model
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Introduction: Alzheimer's disease (AD) is a complex neurodegenerative condition. Recent

research has linked metabolic diseases, such as obesity, to AD, emphasizing the role of decreased glucose metabolism in its pathogenesis.

Objective: Investigating the anti-inflammatory effects of Andrographolide (Andro) on glucose uptake in visceral adipose tissue (VAT) using AD-transgenic mice.

Methods: We used transgenic APP/PS1 mice (4 months of age). The animals were fed a high-fat diet (HFD, 45% energy from fat, purchased from Animal Care, for 4 months) and received periodic Andro injections (2 mg/kg, purchased from Sigma-Aldrich and injected 3 times a week for 16 weeks). VAT samples were collected for various glucose uptake assays. All procedures performed at the P. Universidad Católica de Chile were approved by the Bioethical Committee (150730041). Results were calculated as means ± SEM (n ≥ 3 independent samples) using GraphPad Prism 9. Statistical significance was analyzed with one-way or two-way ANOVA, followed by Bonferroni's post hoc test (*p <0.05, **p <0.01, and ***p <0.001).

Results: Andro reduced glucose uptake in VAT of animals treated with HFD, reversing HFD-induced increases compared to controls. Andro reversed the effect of HFD on glycolysis and glucose metabolic regulators, restoring VAT's metabolic profile, and indicating reduced energy production.

Conclusions: These results reveal the complex relationship between glucose metabolism, AD, VAT, and obesity, indicating Andro's potential to impact VAT glucose metabolism. Andro emerges as a promising therapeutic agent for restoring glucose levels and addressing AD-related metabolic disorders.

Financing: This work was supported by grants from Proyecto Puente UOH (PC), Proyecto Postdoctorado UOH (E.S.) and CIES 007 (P.O.).

P-40

The methyl CpG Binding Protein-2 regulates energy homeostasis and controls the expression of hypothalamic miRNAs through an epigenetic feedback loop

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Introduction: Overweight and obesity are global health problems with increased prevalence, suggesting the contribution of modern lifestyle. This environmental information can be integrated into our genome by epigenetic modifications that modulate hypothalamic gene expression to maintain neural plasticity allowing a proper maintenance of energy homeostasis. Methyl CpG Binding Protein-2, MeCP2, is an epigenetic reader that regulates gene expression through the binding of methylated DNA and control of miRNA processing. Some patients carrying *Mecp2* gene mutations exhibit alterations in body weight, as well as mice lacking a functional *Mecp2*. However, the mechanisms through which MeCP2 regulates energy balance and the impact of modern lifestyle on epigenetic-commanded processes have not been fully elucidated.

Objective: To evaluate the role of MeCP2 in the epigenetic-miRNA feedback system in the hypothalamus and its impact on body weight.

Methods: We used *Mecp2*-null and hypothalamic conditional KO mice in two physiological conditions, fasting and after activating the anorexigenic tone with 4 mg/kg i.p. leptin, in addition to a 12 weeks-HFD-fed wild-type mice (n = 4). The Institutional Animal Care and Use Ethics Committee approved all protocols (14-2020-10).

Results: Mice lacking functional MeCP2 alters the expression of miRNAs (logFC and adjusted p-value) potentially related to the regulation of genes commanding hypothalamic neural plasticity during the anorexigenic tone and in response to an HFD-feeding.

Conclusion: Our results contribute to understand the role of Mecp2 in the hypothalamus as a molecular bridge between environmental factors and our genome required for adequate control of feeding behavior, energy expenditure, and body weight.

Financing: Fondecyt 1181574, Fondecyt 1230905, ANID-ANILLO ACT210039.

P-41 ★*selected for oral communication*

High-fat/low-carb diet induces neural plasticity-associated modifications and changes in epigenetic factors in the arcuate nucleus of the hypothalamus

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Introduction: The prevalence of obesity nearly tripled in the last 40 years, suggesting a contribution of environmental factors, such as changes in feeding habits. The melanocortin system (MS) is a neuronal circuit that requires high levels of plasticity to control energy homeostasis. One of the mechanisms underlying its plasticity is the permanent control of gene expression, a process in which epigenetic pathways play a pivotal role. However, the influence of diet composition on epigenetic modifications in the MS has only been recently addressed.

Objective: To evaluate epigenetic changes that underlie the neural plasticity-related modifications in the MS associated with high-fat/low-carb (HFLC) feeding.

Methods: We used wild-type and transgenic mice fed with an HFLC or chow diet for either 4 or 12 weeks (n = 10). All protocols were approved by the Institutional Animal Care and Use Ethics Committee (14-2020-10). Data is presented as mean ± SEM compared by two-way ANOVA.

Results: We observed that HFLC-fed mice exhibit changes in body weight, food intake, and energy expenditure. In addition, these changes were associated with cytoarchitecture and gene expression pattern modifications of hypothalamic neurons. Interestingly, we observed changes in the expression/phosphorylation of the CpG methyl binding protein (Mecp2), in the expression of miRNAs involved in neuronal cytoarchitecture, and in gene expression associated with sensitivity to metabolic signals involved in energy balance.

Conclusion: Our results highlight the role of chromatin remodeling in the proper hypothalamic function required for adequate control of feeding behavior, energy expenditure, and body weight.

Financing: Fondecyt 1181574 Fondecyt 1230905, ANID-Anillo ACT210039

P-42 ★*selected for oral communication*

Human umbilical vein endothelial cells dysfunction in gestational diabesity

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Introduction: Foetoplacental endothelial function in women with gestational diabetes (GDM) and pregestational obesity, i.e. gestational diabesity, is not characterized.

Objective: To characterize the foetoplacental endothelial function in gestational diabesity.

Methods: Human umbilical vein endothelial cells were from women with normal pregnancies and pre-pregnancy normal weight (N*nw*), overweight (N*ow*), or obese (N*ob* (n = 4-9) and GDM*nw*, GDM*ow*, GDM*ob* (n = 3-5) (Clinical Hospital UC CHRISTUS, with patient consent, Ethics #012793). Cell migration was determined with a wound healing assay (0-24 h). Intracellular pH (pHi) was measured by the acid pulse assay in BCECF-AM (12 µmol/L, 10 min)-preloaded cells. Nitric oxide production was measured by immunofluorescence in DAF (5 µmol/L, 60 min)-preloaded cells ± L-NAME (100 µmol/L, 60 min). All results are presented as mean±S.E.M and were analyzed by two-way ANOVA with $p<0.05$ as significant.

Results: Wounded area recovery (*War*) was lower

in N*ob* (20 ±4%), GDM*ob* (6 ±6%), and GDM*nw* (14 ±2%) versus N*nw* (30 ±4%). *War* was lower (~43%) in GDM*ob* versus GDM*nw*. Basal pHi was higher in N*ob* (7.57 ±0.14), GDM*nw* (8.17 ±0.16), and GDM*ob* (7.74 ±0.15) versus N*nw* (7.06 ±0.06). There were no differences in cell buffering. The *dpHi/dt* in GDM*ow* (0.015 ±0.0022 pHi units/s) was higher (1.7–2.5-fold) than all other groups. Nitric oxide production was elevated in cells from N*ob* (2.1-fold) and GDM*nw* (5.1-fold) compared with N*nw* (1.02 ± 0.30 FRU/µg protein). **Conclusion:** Gestational diabesity modulates migration involving intracellular alkalization without affecting the nitric oxide production in HUVECs.

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AREA: IMMUNOLOGY AND CANCER

P-43 ★*selected for oral communication*

Cytokine profiling in workers at high altitude subjected to chronic intermittent hypoxia

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Introduction: In Chile, main mining activities are developed at altitudes over 3,000 masl. Most workers commute rapidly to high altitude facilities, exposing them to chronic intermittent hypoxia (CIH), whose long-term effects are unknown. Previous works show that CIH elicits a proinflammatory state, associated to TNFα and IL6, but there is discrepancy on other cytokines involved due to different protocols in animal and human models of hypoxia.

Objective: To perform a qualitative assessment of 12 cytokines in blood samples from mining workers from 0 to 4,500 masl.

Methods: Male volunteers with rotating shifts (7d working/7d resting) were recruited at different altitudes (Ethics Committee of Faculty of Medicine CECFAMED #01/09). Physical evaluation was performed at third day of shift, fasting venous blood sample was obtained and 12 cytokines were evaluated (Multi-Analyte ELISArray). Data are shown as percentage of people expressing cytokines from population at different altitudes. No statistical comparison was performed between altitudes.

Results: From 38 samples analyzed, highest presence cytokines were IL8 (61%), IL10 (55%) and IL17A (61%). Regarding distribution by altitude, IL8 and IL10 expressed in 100% of samples from high altitude (N=9), while IL12 and IL6 expressed in 67% and 56%, respectively. IL17A expressed in 100% (N=14) at moderate altitude and only 44% above 3,000 masl.

Conclusion: These data suggest variable presence of several cytokines at high altitude, but further studies are needed to determine them quantitatively, as well as to determine if they contribute to acclimatization response or to appearance of other alterations associated with CIH exposure.

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P-44 ★*selected for oral communication*

Modulation of the immune response by a bacterial consortium applied in mice infected with *Salmonella typhimurium*

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Introduction: Intestinal infections are an important cause of morbidity, in Peru they account for 6% (MINSA, 2023. https://www.minsa.gob.pe); and pharmacological therapy can alter the balance of the intestinal microbiota and the body; therefore, the application of bacteria result in a treatment alternative with probiotic potential, which could favor the host's defense.

Objective: Evaluate the effect of *Bacillus licheniformis* and/or *Lactococcus lactis* on levels of interleukin-6 and interleukin-10 of *S. typhimurium* infected mice.
Methods: The study made up of 32 BALB/c mice, who were divided into four treatment groups (n=8) and were inoculated with: G1: *Lactococcus lactis*; G2: *Bacillus licheniformis*; G3: *L.lactis* and *B.licheniformis;* G4: negative control. After the third day of treatment, all mice were inoculated orally with *S.typhimurium*. At the end of the week, the subjects were sacrificed (Favorable opinion 032-2023, UCSM Institutional Research Ethics Committee), a sample of blood serum was collected for determine the levels of interleukins-6 and -10. The values of means ± S.E.M. were evaluated by ANOVA and Scheffe differentiation test (alpha 0.05).
Results: Mice treated with *L. lactis* and *B. licheniformis* had statistically significant lower serum level of interleukin-6 (mean=49.19 pg/mL) than mice from other groups. Mice treated with *L. lactis* and *B. licheniformis* (mean=203.74 pg/mL) and those treated with *B. licheniformis* (198.22) had significantly higher serum level of IL-10 than another groups.
Conclusion: The application of the consortium *Lactococcus lactis* and *Bacillus licheniformis* would have an immunomodulatory effect in BALB/c mice challenged with *Salmonella typhimurium*, stimulating a decrease in the serum level of IL-6 (proinflamatory) and an increase in IL-10 (anti-inflamatory).
Financing: The source of financing for the development of this study was the Catholic University of Santa Maria.
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P-45

Nrf2 transcription factor and acquired resistance of breast cancer cells against chemotherapeutic drugs. A search for potential therapeutic target genes

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Introduction: Nrf2 (nuclear factor erythroid 2-related factor 2) is a pleiotropic transcription factor that regulates the expression of numerous genes and protects healthy cells from various toxic compounds. However, Nrf2 could protect cancer cells through alterations of its signaling pathway, changing from its transitory to a permanent activation.
Objective: To gain insights regarding the Nrf2-mediated chemo-resistance acquisition by cancer cells and to identify the proteins involved in such protective mechanism.
Methods: Nrf2 was activated by genetic inactivation silencing its repressors using siRNAs (Cullin-3 and Keap1) or exposing cells to tert-butylhydroquinone (tBHQ). To check activation, NQO1 activity and GSH levels were assessed in MCF-7 human cancer cells. Survival was monitored in cells challenged with anticancer drugs: doxorubicin, mitoxantrone, cisplatin, and melphalan. The data were examined using an unpaired two-tailed t-test, using GraphPad Prism software and results are expressed as mean values ± SD (n=3). In addition, a preliminary microarray was conducted in cells treated by tBHQ and siRNA against Keap1 and Cul-3 in order to identify potential candidates involved in cancer cells resistance.
Results: Treated cells were significantly more resistant to different chemotherapies than control cells, with a survival of 70%. (IC50: 3.06 ± 1.02 and 0.93 ± 1.29 respectively). The preliminary microarray gave a list of 19 up-regulated genes common to three Nrf2 activators, among them 15 are known Nrf2 targets.
Conclusion: Nrf2 status is determines cancer cell sensitivity to chemotherapy, where some genes could be candidates involved in mechanisms by which Nrf2 would exert its cytoprotective effect in cancer cells.
Financing: This research was funded by Fondo Nacional de Ciencia y Tecnología (FONDECYT), grant number 1190577, Chile.

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The regulation of microglial phagocytic process by the cGAS/STING signaling pathway

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Introduction: Microglia, the immune system cell in the brain, serves as a guardian through phagocytosis, protecting against infections (bacteria, viruses, and parasites), processing apoptotic cells and cellular debris, and contributing to proper neurodevelopment. The cGAS/STING signaling pathway, which recognizes endogenous and exogenous (bacteria and virus) double-stranded DNA (dsDNA) within the cytoplasm, triggers the release of proinflammatory cytokines and an inflammatory response.

Objective: The purpose of this study is to investigate the potential involvement of the cGAS/STING pathway in microglial phagocytosis.

Methods: Neonatal and young mice (3 months old) were employed, including Wild-Type (WT) mice, mice lacking the cGAS protein (cGASKO), mice lacking the STING protein (STINGKO), and mice lacking both proteins (2KO) (bioethics committee approved code 5/2018, Fondecyt 11190258). The transcriptomics of young mouse brain tissue were assessed via Single Cell analysis. Cytoskeletal and microglial morphology were analyzed using primary cultures of neonatal mice from WT, cGASKO, STINGKO, and 2KO, employing immunofluorescence. The phagocytic capacity of LPS-stimulated microglia was assessed using primary cultures of neonatal microglia WT, cGASKO, STINGKO, and 2KO mice, also through immunofluorescence. Statistical analyses were conducted using One-Way Anova with n=3-5 mice per experimental condition, and results were reported as mean ±SEM.

Results: We demonstrated that cGAS/STING is primarily expressed in microglia. Additionally, we discovered that microglia cGASKO, STINGKO, and 2KO present an altered cytoskeleton and morphology, along with an increased phagocytic capacity.

Conclusion: Our findings indicate that cGAS/STING is involved in the process of phagocytosis in microglia, as well as in inducing morphological changes.

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AREA: LIPIDS AND NUTRITION

P-47

Development of novel Lox-1 inhibitors for potential therapeutic applications

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Introduction: Atherosclerosis, a chronic inflammatory disease, involves complex interactions among modified lipoproteins, immune cells, and vascular components. Elevated oxidized low-density lipoproteins and the activation of the receptor Lox-1 play a key role in vascular dysfunction.

Objective: This study aims to identify molecules that can interact with Lox-1 and evaluate its activity in vitro experiments.

Methods: Using the Receptor Grid Generation tool in Schrödinger's Glide software, we created an interaction grid focusing on specific residues (basic spine, R229, R231, R248). We generated a molecule library from the ZINC15 database, ensuring desirable properties, minimized conformations, and protonation states. These files were used for protein-ligand docking in the same software. Subsequently, we selected molecules with the best binding affinities and favorable parameters in human cells.

Results: The six selected molecules display their QikProp-predicted properties, emphasizing appropriate binding free energy (ΔG) and pertinent pharmacological parameters. The primary interactions reveal a positively charged cavity with hydrogen bonds formed among series 198, 162, 199, 160, glutamate 192, and arginine 248. The most noteworthy interactions involve hydrogen bonds with residues R248, S162, S160, and π-π stacking with F200 and F261. These

molecules are non-cytotoxic and inhibit oxLDL incorporation.

Conclusion: The use of in silico tools allows for the identification of new molecules that bind to the basic spine of Lox1. The candidate molecules exhibit highly favorable energy parameters for predicting receptor binding. The residues involved in the interaction of the candidate molecules with Lox-1 are the same as those found in other inhibitors described for this receptor

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P-48 ★*selected for oral communication*
★ *undergrad sci competition*

Acquired long QT syndrome caused by hERG Potassium channel block in isolated hearts of Cavia porcellus (guinea pig) and its reversion by membrane lipid replacement with nanomicelles

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Introduction: Long QT syndrome (LQTS) is the prolongation of cardiac depolarization, leading to an increase in the QT interval on the electrocardiogram. It is usually related to alterations in the normal functioning of cardiac ion channels either genetically determined or acquired. It can result in serious cardiac dysfunction leading to ventricular arrhythmias.

Objective: The main objective of this work, promote LQTS in isolated hearts and cells from *Cavia Porcellus* using a specific blocker of hERG channels, dofetilide (DFT), to later test if it could be reversed by membrane lipid replacement with nanomicelles with phosphoinositosides.

Methods: A Langendorff coronary retro perfusion device was used to perfuse the heart with Tyrode solution (1.8 mM), while different agents (nanomicelles and DFT) were introduced through separate tubing sets. Contractile activity (measured through a tension transduction system) and electrical activity (measured with AgCl2 electrodes) of the heart were recorded. Both signals were processed and visualized using Axoscope 9.2 software, and analyzed by fitting a standard Hill equation f= min + (max-min) / 1+10^(log EC50-x). Ethical approval was obtained by CHEA (Comisión honoraria de experimentación animal) (protocol 070151-500060-21).

Results: Paired-samples-T-test, mean±S.E.M. The application of ultrasonicated nanomicelles before (n=3) or after (n=3) DFT administration showed a significant shift in the DR curves towards higher DFT values, suggesting that nanomicelles have a preventive or therapeutic effect in treating hERG-induced LQTS.

Conclusion: This represents an innovative pilot experience for preventive and/or therapeutic treatments of LQTS through nanomicelle application and suggests that hERG DFT blockage may be prevented through this nanomicelles.

Financing: Comisión Sectorial de Investigación Científica (CSIC) (Universidad de la República) to GF.

AREA: NEUROPHYSIOLOGY

P-49

Functional expression of inhibitory glycinergic neurotransmission onto somatostatin positive neurons in the ventral pallidum

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Introduction: Although traditionally thought to act primarily in the spinal cord, emerging evidence support important inhibitory functions by glycinergic transmission in the brain. In the ventral pallidum (VP), glycinergic afferents from the brainstem make synapses onto cholinergic interneurons to regulate arousal. Notably, VP is comprised of a heterogeneous population of GABAergic neurons, of which those that express somatostatin (SOM+) comprise a significant group and have been implicated in the control of sleep and wakefulness directly inhibiting cholinergic, glutamatergic and parvalbumin neurons.

Objective: Here, we investigate whether glycine may mediate inhibition onto SOM+ cells to control VP function and circuit communication.

Methods: Using genetic, pharmacology, and electrophysiological approaches, we evaluated the inhibitory synaptic transmission onto somatostatin positive (SOM+) neurons in mice,

approved bioethics act code CBC 55-2022.
Results and conclusion: We found that in addition to GABAergic inputs, SOM+ neurons also receive functional glycinergic inputs, demonstrated by the presence of glycine-induced currents that are sensitive to the blockade by strychnine, a glycine receptor antagonist. Consistently, bath application of strychnine reduces both spontaneous and electrically evoked inhibitory postsynaptic currents (IPSCs) in SOM+ neurons. Moreover, inhibition of the glycinergic transporter (GlyT2) also reduces IPSCs recorded from SOM+ neurons, strongly suggesting the presence of functional glycinergic synapses in the VP. Interestingly, inhibitory synaptic transmission onto SOM+ neurons express activity-dependent changes of synaptic efficacy in the form of long-term depression and can be regulate by cannabinoid signaling in a CB1 receptor dependent manner. Whether these changes in synaptic efficacy could impact both glycinergic and GABAergic synaptic transmission is currently under investigation.
Financing: This work was supported by Fondecyt #1201848 (A.E.C), and by ANID Millennium Science Initiative Program (P09-022F to C.Q.C and A.E.C). A.A. was supported by PhD fellowship from ANID # 21202136 and PhD program in Neuroscience at the University of Valparaíso.

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Heart Rate Variability during short-term head-down tilt

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Introduction: The head-down tilt (HDT) technique can imitate the immediate effects of microgravity. A change in HRV (Heart rate variability) results from the redistribution of fluid from the lower to the upper body caused by HDT. However, the effect of short-term HDT with various degrees on HRV is not well documented and needs to be explored.
Objective: To evaluate the effect of short-term HDT at 6°, 15°, and 30° on HRV in healthy adults.
Methods: After approval from the Institutional Ethics Committee (AIIMS/IEC/2021/3551), we enrolled 50 healthy subjects (Age = 30.38±6.63 (mean ± SD) years). Short-term HDT for 5 minutes was administered using a motorized tilt table with continuous recording of Lead II ECG and chest movements. HRV was analyzed during baseline and each degree of tilt. Values are expressed in the median (interquartile range) and Friedman test was applied.
Results: We observed that the mean RR interval was increasing in all three degrees of tilt compared to the baseline. It was observed that as compared to baseline, there is increase in SDNN(ms) (BL: 39.54(23.14-93.06); HDT = 6°: 48.12(22.44-85.74); 15°: 45.73(21.03-107.2); 30°: 46.02(18.92-106.4); p-value =0.0185*) and RMSSD(ms) (BL: 31.74(14.50-106.9); HDT = 6°: 40.81(11.56-128.5); 15°: 39.73(12.94-119.4); 30°: 38.78(11.33-104.5); p-value =0.0241*), which is suggestive of parasympathetic stimulation during tilt. Total power also showed a significant increase with each degree of tilt, which is representative of an increase in overall autonomic reactivity during tilt.
Conclusion: Short-term HDT leads to increased HRV due to improved autonomic reactivity during tilt with significant involvement of parasympathetic activation.
Financing: No external funding is obtained for this project.
Acknowledgments: All the participants and the Autonomic Function Testing Laboratory, AIIMS Jodhpur staff.

P-51

Physical activity (PA) levels and their correlation with cardiac, biochemical and memory parameters in higher education students

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Introduction: Physical activity (PA) confers widely documented health benefits, especially at the cardiovascular (CV) level. Recently, it has been

evaluated whether PA would have effects on higher functions of the nervous system, such as cognition and memory. Exerkines, such as brain-derived neurotrophic factor (BDNF), which participates in memory, learning and synaptic plasticity, correspond to organ-specific molecules that respond to FA levels.
Objective: To evaluate the response to PA levels (steps) on plasmatic biochemical elements, BDNF and various types of memory in higher education students.
Methodology: We perform a observational study in students (n=36) using principally linear correlation analyzes (Pearson, r) carried out from the records of physiological parameters (PA and CV), through the use of sports watches. Additionally, we measured anthropometric variables, clinical signs, biochemical and BDNF plasma determinations, application of cognitive tests and psychological self-assessment instrument, such us DASS-21 (bioethical approval certified n° 8.30.22).
Results: We confirm the impact of PA on BDNF levels (r=0.89) and resting heart rate (r=–0.39), which also shows a high association with cardiorespiratory fitness (eVO2max, r= –0.72). Although our data do not support a direct effect of BDNF on cognitive performance, other physiological parameters such as grip strength and biochemical parameters such as insulin, showed significant associations with sequential memory (r=0.85 and r=–0.84).
Conclusions: Our data associate PA levels with cardiorespiratory fitness, which would impact plasma parameters such as BDNF and insulin, which in turn, could constitute a possible molecular mechanisms associated with cognitive performance.
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P-52

Single-nucleus RNA-Seq revealed the core genes related to the magnocellular neurons activity

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Introduction: The hypothalamic magnocellular neurons (MCNs) are essential for hydromineral and reproductive regulation due to their ability to synthesize and release the neuropeptides vasopressin and oxytocin. When released, those neuropeptides can act peripherally to control renal water reuptake and milk ejection and regulate several behavioral responses centrally.
Objective: To reveal the genes related to the activity statuses of the MCNs.
Methods: Wistar Hannover adult male rats (Bioethics Committee UK:PPL-PP9294977,n=10) were divided into control and 48 hours of water deprivation. The brains were collected, and a micropunch was performed to collect the hypothalamic supraoptic nucleus (SON). Isolated cell nuclei were processed in a 10XGenomics Chromium-Controller to obtain cDNA libraries for single nucleus RNA sequencing. Libraries were sequenced (Novaseq6000, Illumina), and the data was aligned in CellRanger. Cluster identification was performed in Seurat, and the Neuroestimator predicted the neuronal activity. Correlation analyses were made by Spearman's correlation and was significant when r>0.1 and p<0.05.
Results: Our snRNAseq of the SON identified ~12k of MCNs. The Neuroestimator revealed 411 genes (48 positive and 364 negative) correlated with the MCN's predicted activity. Among those genes, 24 are coding transcription factors, 31 enzymes, 9 GPCRs, and 13 channels. The gene ontology analyses revealed 370 enriched pathways (p<0.05), including the sensory perception of a mechanical stimulus and social behavior. The top positively correlated genes were *Pde3a, Oacyl, Giot1, Pde10a* and *Creb3l1*.
Conclusion: Our findings contribute to understanding the genes related to MCNs activation, offering new candidates to study the mechanisms involved in regulating those neurons under physiological and pathological contexts.
Financing: CAPES: #001; FAPESP 2019/27581-0 and MRC: MR/W028999/1.
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P-53

Physiological response during social cognition tests in subjects according to anxiety and depression symptoms

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Introduction: Social cognition is a relevant area of functional declining in anxious and depressive patients. Nevertheless, anxiety an depressive symptoms are commonly seen in healthy young individuals. We recruited 21 healthy subjects aged 17-25 years.

Objective: The primary objective was to examine how variations in social cognition and working memory performance relate to psychological and physiological markers, specifically heart rate and pulse, in young adults.

Methods: Participants underwent assessments for social cognition and working memory, alongside concurrent monitoring of physiological variables. Additionally, symptomatology of anxiety and depression was evaluated using the Goldberg questionnaire. Statistical analysis was performed with spss software. Mean+SD are given. Pearson correlation was used to evaluate the correlation between continuous variables. The subjects gave informed consent. The protocol was submitted to the ethics committee of the Faculty of Medical Sciences, Bernardo O'Higgins University (code 0303456).

Results: The average social cognition score was 11.5±3. We observed a significant correlation between performance in social cognition tests and scores obtained in verbal fluency (Pearson Coefficient 0.71) and visual learning tests (Pearson Coefficient 0.73). Preceding failures in Theory of Mind tasks, significant alterations in respiratory and cardiac frequencies were observed. Notably, individuals scoring higher on the Goldberg questionnaire, indicative of heightened anxiety and depressive symptoms, exhibited more pronounced physiological disruptions.

Conclusions: These findings underscore the intricate interplay between social cognition, visual memory, verbal fluency and physiological responses in young adults. The association between heightened symptomatology and physiological dysregulation suggests potential avenues for further research in understanding the underlying mechanisms of cognitive and emotional processes.

P-54

Impact of BIST-23, a pharmacological promotor of dynamin´s GTP-ase activity, on the cognitive function, actin organization and dendritic spine dendity in APP/PS1 mice, a murine model of Alzheimer's Disease

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Introduction: Alzheimer's disease (AD) is the leading cause of dementia in elderly. The loss of cognitive function in AD correlates with the loss of functional synapses, due to a decrease in dendritic spines. These are actin-enriched protrusions, which concentrate most of the synaptic contacts in the mammalian brain. Defective actin-remodeling seems to be the main cause of the loss of dendritic spines in AD. Therefore, actin dynamics has emerged in the recent years as a potential therapeutic target. However, a direct intervention of the cytoskeleton is potentially cytotoxic. An alternative is modulate the activity of cytoskeleton-regulator-proteins such as dynamins.

Objective: We evaluated the impact of pharmacologically promoting dynamin´s activity with BIST23 on cognitive function, dendritic spine density and actin organization in brains of APP/PS1 mice, a murine model of AD.

Methods: 6-month-old APP/PS1 and wildtype mice were treated for 14 days with a 10 mg/kg-daily-dose of BIST-23 or its vehicle DMSO. Spatial and recognition memory were assessed by Morris-Water-Maze and Novel-Object-Recognition tests. Dendritic spine density was analyzed in Golgi-stained pyramidal neurons and the relative amounts of filamentous actin (F-) and monomeric actin (G) was analyzed by western blot. Data were expressed as mean +/-SEM. Statistical-differences were tested using ANOVA. $p<0.005$ was considered significant. N=at least 3-mice per

experimental condition. Protocols followed Bioethical standards (BEA02-2022).

Results: The treatment with BIST-23 significantly prevented the recognition and spatial memory loss and increased the F/G ratio in the brain of APP/PS1 mice.

Conclusion: These data strongly suggest that BIST23 promotes F-actin polymerization, preventing the F/G actin imbalance and the memory loss in AD.

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P-55

Effect of aerobic exercise on the endocannabinoid-mediated stress control and reward system in the brain: a narrative review.

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Introduction: Aerobic exercise is widely adopted for its capacity to elevate mood and assist in alleviating depression and anxiety disorders. Endocannabinoids (eCBs) have emerged as credible agents accountable for this mood enhancement.

Objectives: This study aims to amalgamate present insights concerning the eCB-mediated regulation of stress and the brain's reward mechanism ensuing from participation in aerobic exercise.

Methods: A thorough investigation was conducted across databases encompassing Medline, SPORTDiscus, Pubmed, and Scopus, encompassing data available until June 30, 2023. The comprehensive searches culminated in the retrieval of a total of 156 studies, of which 91 underwent eligibility screening, resulting in the inclusion of 53 pertinent studies within this review.

Results: This review underscores that subsequent to a session of moderate-intensity aerobic exercise, circulating cortisol and eCB levels - N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) - notably escalate. A certain threshold exercise intensity is requisite to invoke a cortisol response. Cortisol incites eCB secretion, which reciprocally restrains the hypothalamus-pituitary-adrenal (HPA) axis, as well as the limbic and sublimibic brain structures. Endocannabinoids amplify baseline dopamine levels via the type 1 cannabinoid receptor (CB_1R)-dependent mechanism within specific brain regions.

Conclusion: a) Aerobic exercise orchestrates stress modulation through a negative feedback loop, influencing both the sympathetic nervous system and the HPA axis. b) eCBs assume a pivotal role within cerebral reward mechanisms, predominantly via CB_1R distributed across diverse cerebral centers. c) eCBs partake in inherent reward processes by synergistically interacting with the dopaminergic reward system. d) Genesis of this reward pathway emanates from the ventral tegmental area.

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P-56

Early alcohol exposure induces gene expression dysregulation and long-lasting cognitive impairment

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Introduction: Fetal alcohol spectrum disorder (FASD) is associated with intellectual and executive disabilities and comorbidity with attention deficit and hyperactivity disorder in 50% of children diagnosed with FASD. Moreover, FASD patients have a high vulnerability to developing drug addiction. The reported prevalence of FASD is 5% in the U.S. and 17% in the U.K. The mechanism of alcohol - induced neurodevelopmental reprogramming is not understood. Alternative splicing increases the complexity of the neuronal proteome and therefore regulates the establishment and maintenance of neuronal development and synaptic plasticity. Bioinformatics studies from our laboratory reveal that alcohol exposure modifies the expression of splicing variants (SV) of genes whose protein has synaptic and post-transcriptional regulation functions, both in humans and animal models. We developed a FASD animal model and described cognitive impairment associated with changes in the expression of synaptic genes involved in glutamatergic transmission.

Objective: we tested whether early alcohol exposure changes the splicing factors (SF) and the inclusion rate of (micro) exons in the FASD offspring neonates.
Methods: mRNAs extracted from the prefrontal cortex and hippocampus of 10 Sprague Dawley rats exposed or not to alcohol were analyzed by RT-qPCR, DNA-PAGE, and rqfPCR (approved by CECFAMED-UCN 37/2021, and 15/2023).
Results: We found that acute alcohol exposure modifies the expression of SFs and SVs depending on the brain nuclei and the time after alcohol exposure analyzed. Future studies will focus on understanding the role of SV of synaptic genes in long-lasting alcohol-dependent cognitive alterations.
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P-57 ★*selected for oral communication*
Functional role of the K+ channel Kir7.1 in the transepithelial transport of choroid plexuses
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Introduction: The choroid plexus epithelium (CPE) is responsible for the production and regulation of cerebrospinal fluid (CSF) ion composition. Ion transporters and channels maintain the potential difference between the luminal and basolateral sides required for secretion. Although there is information about the secretion mechanism, the possible role of apical membrane inward rectifier Kir7.1 K^+ channel has not been elucidated.
Objective: To determine the functional role of Kir7.1 in the CPE.
Methods: Tamoxifen-induced conditional knock-out Kir7.1$^{flox/flox}$ TAM mice and vehicle-injected control littermates were used (approved by CECS Ethic committee on 27/01/2022). K^+ currents in CPE cells were studied using patch-clamp whole-cell recordings. CSF ion concentrations were measured by atomic absorption spectrometry in samples from the cisterna magna. NKCC1 transport function was studied by fluorescence microscopy using calcein. Data presented as a mean+SD (n>3) and evaluated by t-test.
Results: CPE-cells of Kir7.1$^{flox/flox}$TAM mice show a significant decrease in Kir7.1-mediated K^+ currents vs control animals (-160mV: -0.37±0.05vs-2.15±0.55 nA; P=0.0002) leading to a depolarization (E_{rev} -52±3 vs -77±2mV; P<0.0001). This was associated with a decrease in CSF K^+ concentration (2.5±0.2 vs 2.9±0.17mM; P=0.0004). There was also a concomitant decrease in the activity of NKCC1 whose ability to increase cell volume was markedly diminished (-4.7±3 vs 25.76±4.62%; P<0.0001).
Conclusions: Conditional inactivation of Kir7.1 leads to a significant loss of K^+ conductance and to a depolarization of CPE cells. K^+ concentration is decreased in the CSF of Kir7.1 KO mice. The absence of Kir7.1 strongly inhibits the function of NKCC1 triple cotransporter in CPE.
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Characterising the effect of menstrual phase on cerebral blood flow control
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Introduction: There is currently little consensus on whether oestrogen and progesterone levels throughout the menstrual cycle affect components of cerebral blood flow (CBF) control. CBF is critical for brain health and is maintained through robust regulatory mechanisms: Neurovascular coupling (NVC), Cerebrovascular reactivity (CVR) and Cerebral autoregulation (CA).
Objectives: This study aimed to characterise the effects of circulating sex hormones on NVC, CVR and CA in pre-menopausal women.
Methods: Ethical approval was provided by the clinical research ethics committee (CREC; ECM 3(r) 14/02/2023) at University College Cork. 15 healthy, eumonnheroic females were tested during the follicular (days 7-10) and luteal (days 19-21) phase of a single menstrual cycle. Participants were free of hormonal contraception. Cerebral blood velocity was measured through the middle and posterior cerebral arteries (MCA and PCA respectively) using transcranial doppler

ultrasound. An intermittent checkerboard stimulus was used to assess NVC response magnitude. CVR index (CVRi) sensitivity was assessed via inspired hypercapnic gas mixtures (2%, 4% and 6% CO_2 with 21% O_2 and balance N_2). Rapid release of bilateral thigh cuffs was used to determine CA responsiveness.
Results: Two-way ANOVA found no significant menstrual phase effect for NVC response magnitude ($p>0.05$), CVRi sensitivity ($p>0.05$) or CA responsiveness ($p>0.05$). Our preliminary results demonstrate stability in NVC, CVR and CA function when comparing follicular and luteal phases.
Conclusions: Collectively, these observations suggest that phasic differences in menstrual cycle is not a mitigating factor when conducting CBF research in lab- or field-based research.
Financing: Funding was provided by the Department of Physiology, University College Cork
Acknowledgments: We would like to acknowledge all participants who graciously volunteered their time throughout this investigation. In addition, we would like to thank the Department of Physiology, University College Cork for funding this investigation.

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The phytoestrogenic effect of Hibiscus sabdariffa involves estrogen receptor α in ovariectomized Wistar rats

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Introduction: *Hibiscus sabdariffa* has multiple pharmacological effects attributed to its high anthocyanin content; however, little is known about its phytoestrogenic properties. The depletion of ovarian hormone production characterizes ovarian hypofunction (OH). OH affects the patients' wellness as estrogens play various functions at reproductive and cognitive levels. Hormone replacement therapy can compensate for OH; nevertheless, long-term use may present adverse side effects. Therefore, natural compounds like anthocyanins, may affect the expression of estrogen receptors (ER) and have potential as phytoestrogens, due to their structural similarity to endogenous estrogens.
Objective: To evaluate the effect of *H. sabdariffa* extract (HSE) on hippocampal ERα and ERβ expression in a murine OH model.
Methods: Ovariectomized Wistars rats received HSE (HSE_{50}:50mg/kg; HSE_{100}:100 mg/kg) or a physiological dose of estradiol for 42 days, complying with the Mexican Official Norm NOM-062-ZOO-1999 and University of Guadalajara Animal care committee (140623-002). ER mRNA and protein were evaluated by RT-qPCR and Western blot. Data are presented as mean±S.D. (n=4), and significances were assessed by one-way ANOVA.
Results: ERα and ERβ are co-expressed in the hippocampus of Wistar rats. Estradiol administration increased the expression of ERβ protein in ovariectomized compared to intact animals; meanwhile, HSE ingestion (50mg/kg) doubled the expression of ERα mRNA. The effect of HSE was dose-dependent. These data coincide with previous reports on short- and long-term memory and hippocampal brain-derived neurotrophic factor (BDNF) in this model (Lorenzana-Martínez et al., 2022).
Conclusion: One of the HSE action mechanisms in ovariectomized rats may involve the phytoestrogenic effect of its anthocyanins, acting as ligands to ERα.
Financing: Funding Research Promoting Programs of the University of Guadalajara, Mexico, supported this work. The first author received a Ph.D. grant from the Consejo Nacional de Ciencia y Tecnología (CONACyT) Mexico.

P-60 ★*selected for oral communication*

The astrocytes from the supraoptic nucleus are activated by hypertonic solution and contribute to magnocellular depolarization

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Introduction: One of the most important areas involved in the body fluid balance is the supraoptic nucleus (SON), in the hypothalamus. Because of its characteristic of intrinsically osmosensitivity, this nucleus has been used as a model of osmoreception. More recently, it has been shown that SON astrocytes could trigger the osmoregulatory process on SON region and may be involved in the control of magnocellular neuron excitability. However, if astrocytes initiate these responses and how they trigger specialized cellular behaviors related to osmoregulation needs to be more explored.

Objective: To investigate the contribution of astrocytes to the excitability of magnocellular neurons during increases in osmolality.

Methods: Single and double whole-cell patch-clamp recording from mice after sulforhodamine 101 staining under, statistical analyses were means±SEM ANOVA Two Way. Ethical committee FMRPUSP#2019/179.

Results: The hypertonic stimulation depolarized the resting membrane potential (-89± 0.5 mV vs -87 ± 0.6 mV, n = 14; $p < 0.005$) and increased the membrane conductance of astrocytes (0.02 ± 0.21 nS *vs* 0.95 ± 0.29 nS). Membrane depolarization was not blocked by furosemide, amiloride, TTX or ruthenium red. Double patch recording (neuron and astrocyte) revealed that the depolarization of the resting membrane potential of astrocytes induced by acute hypertonic stimulation precedes the increase in the activity of magnocellular SON neurons (60 ± 10 sec vs 131± 19 sec; n = 6 pairs, $p<0.006$), suggesting that astrocytes could initiate osmoregulatory processes in this nucleus.

Conclusions: Astrocytes from SON respond to hypertonic stimulation and seem to play a role in osmoregulation.

Financing: FAPESP, CAPES

P-61

Palmitate as a modulator of hippocampal neuroinflammation

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Introduction: Obesity, a global epidemic, is associated with chronic diseases. Elevated palmitate levels in obese individuals raise questions about their impact on hippocampal neuroinflammation and brain function.

Objective: To investigate the effect of physiologically relevant palmitate concentrations on hippocampal neuroinflammation and its implications for obesity-related brain function.

Methods: HepG2 cells were stimulated with 100 µM of palmitate for 24 hours. The conditioned medium was used to incubate hippocampal neurons. Nuclear translocation of NF-κB in neurons and microglia and the expression of COX-1 in astrocytes were assessed through immunofluorescence. Statistical analysis included a one-tailed t-test followed by the Mann-Whitney U test (N = 3, p-values: * $p < 0.0344$, ** $p < 0.0144$, and *** $p < 0.0006$). Ethical approval was granted under code BEA 006-2022, University of Valparaíso.

Results: Palmitate-stimulated conditioned medium from HepG2 cells promotes enhanced nuclear translocation of NF-κB in neurons and microglia, as evidenced by a twofold increase in fluorescence intensity measured in arbitrary units (AU). Moreover, there is an elevated expression of COX-1 in astrocytes, with levels rising from 250 to 600 AU.

Conclusion: These findings strongly support the notion that palmitate induces astrogliosis in this context, leading to a low-grade inflammatory response that adversely affects the function of these cells. Consequently, palmitate triggers neuroinflammation in the mouse hippocampus.

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P-62 ★*selected for oral communication*

Neuroendocrine, metabolic and locomotor

adaptations in the L-NAME model of preeclampsia in mice

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Introduction: Recent evidence points to the role of vasopressin (AVP) in the development of pre-eclampsia (PE). However, the exact mechanisms involved in the hypersecretion of AVP and its signaling pathways to induce PE.

Objective: To study the magnocellular neuron morphophysiology changes and AVP signaling in murine models of preeclampsia.

Methods: Firstly, three models of PE (C57BL/6) were compared in mice: L-NAME in drinking water at 0.5 g/L after gestational day (GD) 7.5; salt loading (1.8% NaCl) after GD 7.5; and a single injection of lipopolysaccharide on GD 7.5 (1 µg/kg, i.p.) (CEUA/UNIFESP: 8225220621) to select the best in reproduce foetal growth restriction. Secondly, fluid and food intake, energy expenditure, respiratory exchange ratio (RER), locomotion activity, plasma FLT1, AVP, copeptin, and corticosterone were evaluated. The significance was set at $p < 0.05$ in T-Test or ANOVA, values are represented as mean ± SD, n of 11 per group.

Results: L-NAME reached an average dose of 82 mg/kg/day and was efficient in restricting foetal weight gain (0.76±0.07g vs 0.86±0.13g), decreasing fluid and food intake (2.12±0.23 vs 1.70±0.22, mL/10g) and energy expenditure (0.54±0.08 vs 0.50±0.06), and reducing locomotion on the GD 8.5 compared to pregnancy control. No significant differences were observed in the weight gain and RER.

Conclusions: Oral L-NAME treatment reproduced the pregnancy alterations commonly seen in PE that might be related to the activity modulation of AVP-producing neurons and since a decrease in locomotion was found only on GD 8.5, a new biomarkers dosage will be performed on GDs 8.5 and 13.5.

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P-63 ★*selected for oral communication*

The extracellular matrix protein Osteopontin regulates Aquaporin-4 expression by activating the calcium channel TRPV4 in retinal Müller cells

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Introduction: During intense neuronal activity in the retina, Müller cells are exposed to a hypotonic environment. This leads to cell swelling and activates a regulatory volume decrease (RVD) response, which depends on Aquaporin-4 (AQP4) and Transient receptor potential vanilloid-4 channel (TRPV4) to induce solutes and water efflux. It was reported that Osteopontin (OPN), a component of the extracellular matrix (ECM), may modulate the RVD of Müller cells.

Objective: To study the putative crosstalk of OPN with AQP4 and/or TRPV4 in volume regulation of Müller cells.

Methods: Cell volume, osmotic permeability (P_f) and intracellular Ca^{2+} levels during an osmotic swelling were measured in the human MIO-M1 cell line by fluorescence videomicroscopy. AQP4 and TRPV4 expression was assessed by immunocytochemistry and Western Blot. Data reported as mean ± SEM, Student´s t-test or ANOVA/Bonferroni's *post hoc* test.

Results: During hypotonic shock, OPN reduced P_f ($.10^{-4}$ cm/s, control vs. OPN: 15.6±0.7 vs. 7.7±0.6, n=4-5, $p<0.001$) and RVD at 10 min (%, control vs. OPN: 35±2 vs. 18±2, n=4-5, $p<0.001$) and increased intracellular Ca^{2+} levels (arbitrary units, control vs. OPN: 0.22±0.02 vs. 0.37±0.05, n=4-5, $p<0.001$). These effects were prevented by TRPV4 inhibitor HC-067047. OPN also reduced AQP4 expression at the plasma membrane, without changes in TRPV4 protein levels or localization. AQP4 downregulation was also reversed by treatment with HC-067047.

Conclusion: We propose that OPN modulates cell volume regulation of Müller cells by TRPV4 activation, which leads to AQP4 downregulation. This represents a novel mechanism of regulation of water permeability by the ECM in Müller cells.

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AREA: RENAL AND GASTROINTESTINAL

P-64

The albumin overload promotes a NGAL-dependent renal fibrotic damage in rodents

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Introduction: Increased albuminuria represents a risk factor for morbidity and mortality in chronic kidney disease (CKD), and it's related to the development of inflammation leading to renal fibrosis. In addition, the Neutrophil Gelatinase-Associated Lipocalin (NGAL) is significantly increase in patients and in experimental animals with CKD, positioning it as a biomarker of renal damage. However, it is unknown whether NGAL is necessary for the promotion of renal fibrosis associated to albuminuria.

Objective: To study whether the NGAL expression is necessary for the development of fibrotic damage induced by the albumin-overload (AO) in mice.

Methods: Male C57BL/6J Wild-Type (WT) and NGAL-KO mice (8-12 weeks, n=5-6) were subjected to AO model (10mg/g/day, i.p.) or Vehicle (0.9%NaCl) for 7-days (bioethical approval #BE08-20), where renal function and fibrotic damage were assessed. Data are presented as mean ± S.E.M., and one- or two-ways ANOVA were performed.

Results: AO significantly increased plasma and urine levels of proteins (1.3 and 7-fold, respectively) in WT and NGAL-KO mice.

Creatininemia and blood-urea nitrogen showed no changes in AO-exposed groups. However, the AO increased the interstitial collagen area from 0.55% to 0.95% in WT mice, which was prevented in NGAL absence (P<0.01). This correlated with a renal increase in mRNA levels for collagen-1 and 3 in response to AO, and with an increase for TNF-α and iNOS (P<0.05), two pro-fibrotic mediators. All these changes were prevented in kidneys of NGAL-KO mice (P<0.05).

Conclusion: The NGAL is necessary for the albumin-induced renal fibrosis in experimental animals, apparently, independent of renal function.

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P-65

Inhibition of Class I and IIa HDACs using valproate ameliorates Diabetic Nephropathy-associated fibrosis

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Introduction: Diabetic Nephropathy (DN) is a complication of diabetes being the main cause of end-stage renal disease. During DN the kidney incurs progressive and irreversible fibrosis guided by Transforming Growth Factor-beta (TGF-β), which remains without interventional options. Previous studies in DN models have shown a sustained loss of histone acetylation and enrichment in class I/IIa Histone deacetylases (HDACs) enzymes mainly in glomerular cells, which correlates with proteinuria and podocyte dedifferentiation. Several HDACs inhibitors have shown antifibrotic effects, being Valproic Acid (VPA) one of the most effective.

Objective: In this study, we look to shed light on the epigenetic effects associated to VPA treatment in diabetic rats and in human podocytes and tubular cells lines.

Methods: Streptozoticine-induced diabetic rats were treated with VPA in drinking water for 2 months DN progression was tested in urine, and Kidney histologic preparations (Bioetic Certificate: 432/2021). Protein and mRNA levels of Nephrin and Podocin were also measured. Fibrosis markers were evaluated in human tubular cells and Podocytes, treated with TGF-β and High glucose in VPA presence.

Results: We demonstrated that VPA treatment in DN rats reverted proteinuria and adenosine urine levels. VPA also reverted histone acetylation loss and myofibroblast markers expression such Fn1 and α-SMA. Moreover, transcripts and proteins levels of podocyte-specific genes such as Nephrin and Podocin were strongly improved *in vivo (t-test, p<0,05).*

Conclusion: Our results support the idea that DN-related fibrosis is a "repressive disease" and that the pharmacological Inhibition of Class I and IIa HDACs with VPA is a viable treatment.

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P-66 ★*selected for oral communication*

Mechanistic insight into angiotensin II type 2 receptor (AT2R) nephroprotective effect during renal ischemia/reperfusion

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Introduction: AT2R agonist C21 elicits nephroprotective effects in ischemia/reperfusion (IR)-induced acute kidney injury (AKI) by preventing tubular cell damage. IR-induced AKI is associated with tubular cell deciliation. Primary cilia are sensory organelles, whose stability depends on α-tubulin acetylation. Extracellular signal-regulated kinases (ERK) activate α-tubulin deacetylase HDAC6.

Objective: To get mechanistic insight for C21 nephroprotective effect during IR.

Methods: Male Wistar rats were pretreated 24h with C21 0,3 mg/kg/day and subjected to unilateral renal IR (n=3-5 rats/group; Institutional Animal Care and Use Committee-FBIOyF Resolution #023-2020). C21 effect was also assessed using a serum/ATP depletion model of IR in MDCK cells (n=3-4 independent experiments). C21 or MEK1/2 inhibitors (PD98059 5µM or U0126 10µM) were added to the media 24h before IR.

Cell ciliation, relative cilia levels of acetylated-α-tubulin (c-Ac-αtub) and ERK1/2 localization were analyzed by immunofluorescence microscopy, activated ERK (pERK) by immunoblotting and cell viability by Trypan blue exclusion test. For cilia length, results are expressed as media±S.E.M. ANOVA/Holm-Sidak-test, Kruskal-Wallis/Dunn's-test or t-test were applied. *p<0.05 vs control (C), #p<0.05 vs C-IR.

Results: C21 prevented IR-induced cilia shortening (length (um): C:3.7+/-0.3; C21:3.4+/-0.3; C-IR:2.1+/-0.1*; C21-IR:2.9+/-0.3) and cell deciliation (C-IR:-32%*; C21-IR:-14%#) and increased basal c-Ac-αtub (+38%*) in renal proximal tubules in the rat. C21 also increased c-Ac-αtub in MDCK cells (+34%*), whereas it inhibited ERK1/2 activation (pERK1:-30%*; pERK2:-34%*).ERK1/2 was conspicuously localized at the primary cilia.ERK1/2 inhibitors partially prevented IR-induced decrease in cell viability (C-IR:-34%*,U0126-IR:-19%*#; C-IR:-22%*, PD98059-IR:-14%*#), and increased basal c-Ac-αtub (U0126:+22%*; PD98059:+20%*).

Conclusions: AT2R nephroprotective effect is associated with primary cilia stabilization by inhibition of the ERK1/2 pathway.

P-67 ★*selected for oral communication*

Galectin-8 counteracts acute kidney injury induced by folic acid

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Introduction: Acute kidney injury (AKI) is caused by a variety of damaging conditions including hypoperfusion, sepsis, cardiorenal syndrome, and nephrotoxicity. The tissue repair process involves dedifferentiation and proliferation of surviving tubular cells as part of an epithelial-mesenchymal

transition process (EMT). When this tissue repair process fails to be completed or becomes prolonged, without resolving inflammation, it usually derives in tissue fibrosis and chronic kidney disease (CKD). There is no directed treatment for AKI. Galectin-8 (Gal-8) is a secreted carbohydrate-binding protein that modulate multiple signaling pathways through its binding to a variety of cell surface glycoproteins. In Madin-Darby canine kidney epithelial cells, we previously described that Gal-8 promotes cell dedifferentiation, proliferation and migration associated with EMT. Gal-8 is also immunesuppressor.

Objective and methods: Here we evaluate the role of Gal-8 in kidney repair in a nephrotoxic mouse model of AKI induced by folic acid. Animal experiments were approved by the Ethical committee of Universidad San Sebastián (01-2021-10). One way ANOVA followed by Tukey's multiple comparisons test were performed to analyze the experimental groups. Results are shown as mean±s.e.m., n=5.

Results: We show that intraperitoneal injection of Gal-8, 24 hours before AKI induction, preserves renal function, decreases the expression of tubular damage markers KIM-1 and NGAL, reduces epithelial cell death and ameliorates cortical damage($p<0.05$). Moreover, Gal-8 treatment also reduces the expression and the interstitial detection of fibrosis and myofibroblasts markers($p<0.05$). Similar effects are observed when Gal-8 is injected 24 hours after AKI induction.

Conclusion: These results suggest that Gal-8 treatment may be used to protect kidneys against nephrotoxic AKI.

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P-68

Depletion of CD11c+ dendritic cell prevents the fibrotic status caused by the 5/6 nephrectomy in mice

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Introduction: The antigen-presenting cells (APCs) recruitment and activation at renal level are pivotal mechanisms in the inflammatory stages that drive to fibrosis in chronic kidney disease (CKD). Our previous studies suggest that dendritic cell-type APCs (DCs-CD11c$^+$) play a crucial role in inflammation and renal dysfunction in mice with hypertensive nephropathy. However, their contribution to the profibrotic state in CKD remains unknown.

Objective: To determine whether the depletion of DCs-CD11c$^+$ prevents the pro-fibrotic status in a 5/6-nephrectomy model ($Nx_{5/6}$).

Methods: Male CD11c.DOG mice (8-12 weeks, n=3-7) were depleted of DCs-CD11c$^+$ by using diphtheria toxin (DT, 8ng/g/day, i.p.) and underwent to $Nx_{5/6}$ or Sham surgery for 5-days (Bioethics approval BE01-20). Animals exhibiting >60% loss of DCs-CD11c$^+$ were selected for the experiments. Histological, molecular, and cellular analyses were performed. Data are presented as mean ± S.E.M., and Kruskal-Wallis one-way ANOVA test were conducted.

Results: The $Nx_{5/6}$ led to a tendency for an increasing plasma creatinine and blood urea nitrogen (P=0.16 and P=0.08 vs. Sham-Vh, respectively), promoted renal fibrosis, and induced high mRNA levels of collagens (COL1A1, COL3A1) and Fibronectin-1 (P<0.05 vs. Sham-Vh). Besides, $Nx_{5/6}$ induced pro-inflammatory mRNA markers (NGAL, IL-6; P<0.05 vs. Sham-Vh). DCs-CD11c$^+$ depletion in $Nx_{5/6}$ animals prevented renal fibrosis (3.79%; P<0.05) and the rising mRNA levels of COL3A1 (P<0.05). Also, DCs-CD11c$^+$ depletion was associated with lower COL1A1 and Fibronectin-1 abundance (P=0.07 and P=0.12, vs. $Nx_{5/6}$-Vh, respectively), without affecting kidney pro-inflammatory markers or plasma parameters of renal function.

Conclusion: DCs-CD11c$^+$ depletion prevents, specifically, the profibrotic phenotype of $Nx_{5/6}$ model.

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P-69 ★*selected for oral communication*

NBCe1 KO mice exhibit changes in the mRNA expression of SCL4 family transporters in gastrointestinal system

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Introduction: The Nbce1 (Slc4a4) Na+-HCO3- cotransporter is important for intracellular pHi regulation, HCO3- secretion in epithelia and renal HCO3-reabsorption. Patients carrying mutations in SLC4A4 gene exhibit metabolic acidosis due to loss of HCO3- in the urine. Nbce1 is also expressed in the gastrointestinal tissue, the effects of its absence or malfunction are unclear, suggesting the presence of compensatory mechanisms.

Objective: Analyze the expression of Na+-HCO3- cotransporters Nbcn1, Nbcn2 and Nbce2; Cl-/HCO3- anion exchangers Ae1, Ae2 and Ae3, and the cation dependent Cl-/HCO3-exchanger Ae4 in different tissues in Nbce1-/- mice.

Methods: The expression of SLC4 family mRNA was analyzed using RT-qPCR from kidney (as positive control expression to Nbce1), salivary glands, stomach, duodenum, liver, pancreas, and colon of Nbce1-/-mice and wild-type mice. The Bioethical Committee on Animal Use of UACh (C49-2020) approved the use of the tissue. The expression was analyzed with 2-ΔΔCtmethod. A non-parametric Mann-Whitney statistical was done, using +/-SD with n = 3 for each group.

Results: We found that wild-type mice showed high expression of Nbce1 in the stomach, comparable to kidney tissue, whereas the duodenum and pancreas showed the lowest expression. The stomach of Nbce1-/- mice showed upregulation of Nbce2 and downregulation of Nbcn1, Ae1, Ae2, Ae3, and Ae4. Finally, the expression of Ae1 increased in the liver and decreased in the duodenum.

Conclusion: Our results suggest that the up-regulation of Na+-HCO3- cotransporters and down-regulation of Cl-/HCO3-exchangers might contribute to keep intracellular HCO3- constant because they mediate HCO3-uptake and extrusion, respectively.

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AREA: SKELETAL MUSCLE AND EXERCISE PHYSIOLOGY

P-70 ★*selected for oral communication*

CCL5/RANTES induces a sarcopenic phenotype through a mechanism dependent on NADPH oxidase and NF-kB

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Introduction: Sarcopenia, a syndrome characterized by a decline in muscle strength, mass, and physical function, may be generated by soluble factors such as myokines. CCL5/RANTES is a myokine capable of modulating the NADPH oxidase (NOX) protein complex and the NF-kB transcriptional activity through the CCR5 receptor.

Objective: Evaluated the NADPH oxidase complex and NF-kB participation in the sarcopenic phenotype induced by CCL5/RANTES through the CCR5 receptor.

Methods: C2C12 myotubes and isolated-Flexor digitorum brevis (FDB) fibers were incubated with 1mM of Apocynin (NOX inhibitor) and 100µM of Andrographolide (NF-kB inhibitor) for 1 h before incubation with 200 ng/mL of recombinant CCL5 (rCCL5) for 72 h. Indirect immunofluorescence was performed in fixed cells, and protein extracts were obtained for western blot analysis. The results are expressed as mean ± standard deviation, considering an n=3 for each experimental set, and the t-test and 1-way ANOVA statistical tests were

performed for for treatment with both inhibitors, a value of $p<0.05$ was used to be considered significant.UNAB bioethical committee approval 003/2023

Results: We observed that incubating myotubes or FDB fibers with rCCL5 decreased diameter and the protein levels of sarcomere components. An increase in reactive oxygen species (ROS) and MuRF-1 and atrogin-1 mRNA levels was also observed. Apocynin prevented the ROS increase and partially prevented the decrease in the fiber diameter. In addition, Andrographolide prevented the rise of MuRF-1 and atrogin-1 and prevented the reduction in the fiber diameter.

Conclusion: CCL5/RANTES is a myokine that induces a sarcopenic phenotype by activating the NADPH oxidase complex and NF-kB.

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Concordance between direct and estimated maximum oxygen consumption in postmenopausal breast cancer survivors from the 6-Minute Walk Test

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Introduction: Maximum oxygen consumption (VO2 max) is a widely used health predictor in oncology. However, the concordance of the equations used to estimate VO2 max from the performance in the 6-Minute Walk Test (6MWT) has not been tested in breast cancer population.

Objective: To evaluate the concordance between direct and estimated VO2 max based on the 6MWT in postmenopausal breast cancer survivors.

Methods: VO2 max was directly measured using a portable metabolic analyzer (PNOE) while performing the 6MWT in a group of postmenopausal breast cancer survivors (53±4 years; 5.1±2.4 years post-therapy; n=13). Three equations from Burr *et al., 2011* (A), Mänttäri *et al.,2018* (B), and J. Vásquez-Gómez *et al.,*2018 (C) were used to estimate VO2 max based on participants' characteristics and the distance covered in the 6MWT. Study approved by the ethics committee of the UFRO N°004_23

Results: Bland-Altman plots revealed a mean difference ± Standard Deviation (SD) of 0.95±6.25, 4.79±5.76, and -4.36±7.30 ml/kg/min between direct and estimated VO2 max using equations A, B, and C, respectively. Notably, there were no significant differences only between direct and estimated VO2 max by equation A. The Lin's Concordance Correlation Coefficient (CCC) was poor with $pc \leq 0.09$ in all three analyzed equations.

Conclusion: In situations where direct measurements of VO2 max are unavailable, the A equation may still serve as a valuable tool for estimating VO2 max in postmenopausal breast cancer survivors women, even though it exhibits a poor Lin's CCC. The estimation of VO2 max using

the A equation is the closest approximation to direct measurements.

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Characterization of pyroptotic cell death in a skeletal muscle cell line

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Introduction: Pyroptosis corresponds to a type of "programmed cell death (PCD)", described in recent years in cancer cell lines and other types of tissues. Its importance lies in the fact that it can trigger inflammatory reactions, a process at the basis of a great variety of diseases. How this process is carried out in skeletal muscle is not yet fully understood.

Objective: to answer whether pyroptosis is present in skeletal muscle cells and to characterize this process in C2C12 cells.

Methods: The gastric cancer cell line AGS was used as a cell type where pyroptosis has been previously described. The skeletal muscle cell line (C2C12) was used to study the pyroptotic process. Cells were stimulated with H2O2, cisplatin, or ATP, known for inducing different types of PCD. To confirm cell death, we used propidium iodide. Western blot and immunofluorescence were used to analyze the expression of the protein Gasdermin-D (GSDM-D).

Results: After stimulation for different times with H2O2, cisplatin, or ATP, AGS and C2C12 cell lines, showed to undergo cell death. The protein known as Gasdermin D (GSDMD), a hallmark that is present in pyroptotic death processes, was shown to be present in these cells both in its total form (GSDM-D) and in its active form (GSDM-NT).

Conclusion: Skeletal muscle cell line C2C12 shows to suffer cell death processes with different agents, some of them activating GSDM-D protein, a step characteristic of pyroptosis.

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P-73

Overactivation of the extracellular ATP – interleukin 6 pathway in skeletal muscles atrophied by botulinum toxin

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Introduction: Botulinum Toxin Type A (BoNTA) injection in masseter muscle is widely used as a clinical and aesthetic procedure. However, it leads to muscle atrophy. We have demonstrated that the expression and release of interleukin-6 (IL-6) are induced by the extracellular ATP (eATP) signaling pathway in normal skeletal muscle. This pathway is overactivated in aged or pathological muscles (dystrophic, denervated). In addition, the persistent increase in plasma IL-6 leads to muscle atrophy in several chronic pathologies.

Objective: To evaluate the eATP/IL-6 signaling in mice masseter muscle atrophied by BoNTA-injection.

Methods: Male BalbC mice (8 weeks-old) were injected with BoNTA (0.2U/10µL) or saline solution in the right and left masseter, respectively (IACUC-UChile #20381-ODO-UCH). Masseter muscles were dissected after 2-7-14d. Muscle atrophy was confirmed by histology and molecular markers (Atrogin1, Murf1). ATP release was addressed by luciferin/luciferase assays. mRNA and/or protein levels of the eATP/IL-6 pathway components were evaluated by RT-qPCR and immunoblot, respectively. Results expressed as mean±SEM (n=3-6, $p<0,05$, Wilcoxon-test).

Results: BoNTA gradually evoked masseter atrophy after injection. The resting levels of eATP

were 50%-increased in BoNTA-injected muscles. BoNTA significantly raised mRNA expression of ATP-releaser conduits (pannexin-1, connexin-43) and P2Y/P2X eATP-receptors. The expression of IL-6 and its receptors (IL-6R, gp130) was significantly increased in BoNTA-injected muscles, up to 4.2±0.6, 2.9±0.9 and 6.1±2.2 -fold, respectively. Although the eATP pathway was overactivated, BoNTA-injected muscles became insensitive to exogenous ATP when the ATP-induced increase in IL6 expression was assessed.
Conclusion: The eATP/IL-6 pathway is overactivated and deregulated in masseter muscles atrophied by BoNTA injection.
Financing: Funded by Fondecyt Chile N°1201385.

P-74

Disulfiram promotes the reduction of IL-1β release and increases glucose uptake in skeletal muscle of insulin - resistant mouse

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Introduction: Insulin resistance (IR) is linked to obesity and chronic low-grade inflammation. During IR, increased IL-1β release would promote systemic inflammation and decrease glucose uptake in the skeletal muscle (SM). Disulfiram (DSF) demonstrates the potential for weight reduction in obese mice, yet its influence on glucose regulation remains unclear.
Objective: To evaluate the effect of DSF on insulin resistance and IL-1β release in SM.
Methods: Male C57BL/6 mice were fed a high-fat diet (HFD, 60% Kcal-fat) for 7 weeks and divided into two groups: HFD injected with or without DSF (HFD+DSF, 50mg/kg) for 3 weeks. We determined: i) weekly dietary intake, body weight, glucose tolerance test, insulin levels, plasma IL-1β levels, and IL-1β release from the gastrocnemius muscle, ii) insulin sensitivity through Akt phosphorylation (P-Akt) in the *Flexor digitorum brevis* (FDB) and uptake of fluorescent 2-deoxyglucose analog (2-NBDG) in isolated FDB muscle fibers. Data were analyzed as mean ± SEM. Statistical significance between two groups (Mann-Whitney U test) and the analysis of four groups (two-way ANOVA with Tukey's multiple comparisons test). p-value ≤ 0.05, (n = 5-10). Ethical approval from CICUA, University of Chile, No. 21433.
Results: The HFD+DSF group decreased body weight and improved fasting blood glucose levels compared to the HFD group. Both plasma and gastrocnemius muscle IL-1β release were decreased, and 2-NBDG uptake and P-Akt improved after insulin stimulation in the FDB muscle.
Conclusion: DSF administration significantly improved key physiological parameters in insulin resistance, suggesting its potential as a promising intervention for mitigating this form of metabolic dysfunction.
Financing: Financed by FONDECYT – 1190406/1231103 (Principal investigator: PhD Llanos P.) and ANID Doctoral Scholarship (Cadagan C.).
Acknowledgments: PhD Llanos P. (University of Chile, Chile) - PhD Tapia G. (University of Chile, Chile) - PhD Estrada M. (University of Chile, Chile) - Muscle metabolism laboratory (University of Chile, Chile).

P-75

Chronotype-dependent variations in physiological responses to exercise at different times of day: a preliminary study

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Introduction: The optimization of exercise timing holds significant therapeutic importance to maximize its health benefits. Skeletal muscle exhibits a strong circadian profile, suggesting that coordinating exercise sessions with an individual's circadian rhythms may be an effective strategy to enhance its benefits.
Objective: This study aimed to evaluate how exercise timing impacts physiological responses,

including blood lactate levels, blood pressure, VO_2 max, maximum heart rate, and ventilatory thresholds, among individuals with varying chronotypes.

Methods: Twenty healthy subjects aged 18-30 completed two morning (before 11 AM) and two afternoon (after 4 PM) maximal cardiopulmonary exercise tests (CPETs), with a minimum 48-hours gap between sessions. Specialized equipment measured physiological parameters including resting metabolic rate, lactate concentrations, and cardiorespiratory responses. Statistical analyses involved Wilcoxon signed-rank tests and Spearman correlations (α=0.05). Ethical approval was granted by the Pontificia Universidad Católica de Chile (#220906004).

Results: Preliminary findings revealed significant differences in VO_2 max between morning and afternoon exercise sessions. Ongoing analysis will explore variations related to individual chronotypes, shedding light on the impact of chronotype on exercise responses.

Conclusion: This preliminary study underscores the potential influence of chronotype on physiological responses to exercise at different times of day. The observed differences in VO_2 max suggest that optimal exercise timing may vary based on an individual's circadian preferences. Understanding these variations could have implications for personalized exercise prescription, potentially maximizing the health benefits of physical activity and aiding in the management of metabolic disorders. Further analyses are needed to fully elucidate the extent of these chronotype-dependent effects.

Financing: This research was supported by ANID–ACT210083, Puente-UC 2022-22, and Fondecyt 1230844.

P-76

Assessing quadriceps muscular function after 5 months of anterior ligament reconstruction using surface electromyography and measurement of maximum voluntary isometric contraction, a case report

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Introduction: The anterior cruciate ligament reconstruction (ACLR) involves a prolonged physical therapy (PT) process. Quadriceps dysfunction is common during this, impacting functional activities. Maximal voluntary isometric contraction (MVIC) and Surface Electromyography (sEMG) can give information about muscular function. These assessments are rarely used during rehabilitation process.

Objective: To describe the results of MVIC and sEMG in quadriceps during process of PT after ACLR.

Methods: A 24-year-old male underwent to ACLR surgery. During fifth month of PT, MVIC was performed, using an electronic dynamometer KForce. In sitting position with knee flexion at 90°, dynamometer strap is on the ankle. At the same time, EMG activity of quadriceps was measured with Noraxon wireless sensors in vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF). The patient signed informed consent, and the protocol was approved by the scientific ethical committee (2023-87), Faculty of Medicine, Clínica Alemana-Universidad del Desarrollo.

Results: In the MVIC a strength deficit for the right limb of 22.6% was registered. In sEMG measurement, for the operated right limb, the difference of absolute peak amplitude of the right side respect to the contralateral side was -0.8%, 16.0% and -56.4% in VL, VM and RF, respectively.

Conclusion: The marked asymmetry in MVIC and EMG activity between limbs, and higher peak amplitude values in VL, VM compared to RF could give signs to an effect on generation of peak isometric force in quadriceps. It could be used for PT, promoting exercises that allow greater activation of RF over VL and VF.

Financing: The case report presented had no associated funds, nor did it include extra costs for the patient.

Acknowledgments: Acknowledgements are directed to the Clinica Alemana de Santiago and Universidad del Desarrollo, together with the patient involved, allowed us to use the data and analyze it for this purpose.

P-77

How much skeletal muscle mass do human forearm lose during disuse-induced model? A meta-analysis

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Introduction: Disuse-induced models are considered valid methods to study skeletal muscle atrophy (SMA) in humans. However, there is scarce evidence regarding the magnitude of SMA caused by disuse-induced models in forearm.
Objective: To determine the magnitude of SMA of forearm muscles in humans subjected to experimentally disuse-induced models.
Methods: An electronic search was conducted in eight databases up to August 2023. Studies were included: 1) Performed on healthy humans >18 years. 2) At least one group used only a disuse-induced model with a cast, sling, splint, or another immobilization device for the upper limb. 3) Evaluated SMA with any method. Based on pre-post measurements reported by the studies, fixed or random effects meta-analyses were performed to calculate mean differences (MD) or standardized mean differences (SMD) with 95% CI.
Results: Of a total of 4019 articles, nine studies were included. All studies used cast immobilization on the non-dominant forearm-hand for 3-4 weeks. A significant decrease in immobilized forearm skeletal muscle mass was observed (SMD=0.30; CI=0.11-0.49; P=0.004; I2=0%, P=0.99), equivalent to 0.56-3.52%.
However, the results vary depending on the evaluation method used: forearm circumference (MD=0.17 cm; CI= -0.072-0.42; P=0.17; I2=0%), extensor and flexor wrist muscle thickness (MD=0.12 cm; CI, 0.02-0.22; P=0.016; I2=0%; 0.0053 cm per day), and forearm cross-sectional area (MD=0.09 cm2; CI= -0.52-0.72; P=0.76; I2=0%; 0.0038 cm2 per day).
Conclusion: With a small effect size, the disuse-induced models in humans significantly decreased skeletal muscle mass between 0.56-3.52% in the forearm muscles. The magnitude of the SMA varied depending on assessment method.
Financing: No funding.

Wednesday 29

AREA: EDUCATION

P-1 ★*selected for oral communication*

Investigating the human physiology through smartphone-assisted experimentation: development of a rubric and outcomes of pre-service science teachers

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Introduction: Educational technologies are valuable resources that facilitate physiology education in challenging contexts, such as restricted funding and remote learning. Smartphone-assisted experimentation (SAE) is favored by the popularization of smartphones among students, who are capable of collecting and analyzing body signs anywhere and anytime.

Objective: To develop a rubric for evaluating the academic outcomes of pre-service science teachers exposed to the Investigating the Human Physiology (IHP) activity, an inquiry-based learning activity grounded on SAE.

Methods: Applying the mixed method of educational research, undergraduate teaching assistants were interviewed to validate the rubric, followed by the evaluation of the IHP reports of the students (N=370), approved by the Unifesp IRB (CAAE:69850223.3.0000.5505). Mann-Whitney test and mean±SD were applied.

Results: The sub-items (hypothesis, methodology, results, and conclusion) of the projects were analyzed, as well as the physiology contents and selected variables. Rubric validation revealed substantial to near-perfect agreement (0.623 to 0.906) between the judges in the ICC test. In general, students obtained significantly more good or excellent grades for the majority of sub-items, except for their capability of elaborating a predictive hypothesis (unsatisfactory/regular 46.53±11.51 vs good/excellent 53.47±11.51, p = 0.24) and generating a conclusion from the results (unsatisfactory/regular 50.10±16.18 vs good/excellent 49.90±16.18, p = 0.98). Most projects investigated cardiovascular and nervous system functions aligned with a predominant selection of heart rate and sleep signs as analyzed variables.

Conclusion: Thus, using SAE is promising educational technology to foster inquiry-based learning, evaluate acquiring competencies, and create awareness about mobile health in physiology education.

Financing: ProEDUCA/FAPESP (#2022/06869-8)

P-2 ★*selected for oral communication*

Effect of blended teaching associated with formative assessments on learning about control of blood pressure and on pre-test stress and anxiety

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Objective: The aim was to analyze the effect of blended teaching associated to formative assessments (BT) on blood pressure control (BPC) learning, stress and test anxiety in university students.

Methods: Two freshman classes of a Dentistry course were divided into control (C, n = 50) and BT (n = 49) groups and participated in 3 BPC classes (Institutional Ethics Committee #10859119.0.0000.5418). In class 1, students completed the State-Trait Anxiety Inventory (STAI) to assess anxiety and collected saliva to measure the stress biomarker alpha-amylase. C had 2 lectures. In class 1 of BT, students evaluated, in teams, situations with alterations in BPC. Before class 2, they were requested to see pre-class videos with questions about BPC (Edpuzzle@), and an interactive lesson (Lt-Kuracloud ADInstruments@), with exercises and immediate feedback. In class 2, students answered an individual exercise and the teams discussed which systems of BPC were activated in the same situations from class 1, indicating responses triggered. In class 3, C and BT answered a test and STAI, after saliva collection. Data were analyzed

using t-test for test score and two-way ANOVA + Tukey for anxiety and alpha-amylase.

Results: BT presented higher score on the test (mean ± SD 8.75 ± 0.17) compared to C (7.27 ± 0.26). Before test, C group had higher salivary alpha-amylase (49.69 ± 4.74 U/mL) and higher level of anxiety (55.34 ± 1.53) than BT (41.10 ± 2.45 U/mL; 49.08 ± 1.22).

Conclusion: BT with formative assessments increased learning on BPC, reduced pre-test stress and anxiety.

Financing: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 2019/05987-4 and FAPESP fellowship 2022/12023-4), Fundo de Apoio ao Ensino, Pesquisa e Extensão (FAEPEX grant 2288/20), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq fellowship 141117/2017-6 and 140918/2019-1).

P-3

Student perceptions of the Lt-Kuracloud online platform in both remote and face-to-face teaching

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Introduction: At university, digital teaching strategies are valuable for freshmen adjusting to their new study routines. During the 2020 COVID-19 pandemic, ADInstruments provided free access to Lt-Kuracloud platform's interactive lessons (IL) to Brazilian universities. ILs consisted of explanatory texts, videos, and exercises with immediate feedback. In the Dentistry course at Piracicaba Dental School in Brazil, ILs were offered as supplementary class activities during remote teaching (RT) and also after pandemic, in face-to-face teaching (FT).

Objective: to assess student perceptions of IL usefulness in RT and FT.

Methods: This study was approved by the Institutional Research Ethics Committee (CAAE: 42980515.0.0000.5418). ILs about action potential, muscle contraction, autonomic nervous system, cardiac cycle and blood pressure control were used. Each lesson had a set completion deadline and contributed to the overall subject evaluations. Sixty-six RT and fifty-nine FT Dentistry freshmen from Piracicaba Dental School in Brazil participated, rating the usefulness of IL on a scale of 0 to 5 (very useful) and indicating until 3 justifications for their answers.

Results: There was no difference in the mean response between RT (4.76 ± 0.50) and FT (4.79± 0.46) as determined by Student t-test. The three most frequent justifications provided by the students were that ILs enhanced comprehension, offered an alternative method to study the same subjects and allowed the students to assess their understanding and identify any areas of uncertainty.

Conclusions: In students' view, ILs were beneficial for learning during both RT and FT, enabling teachers to expand virtual teaching strategies and resources.

Financing: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 2019/05987-4), Fundo de Apoio ao Ensino, Pesquisa e Extensão (FAEPEX grant 2288/20).

AREA: GENERAL

P-4

Discriminant model for insulin resistance in type 2 diabetic patients

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Introduction: Patients with type 2 diabetes mellitus tend to have insulin resistance, a condition that is evaluated using expensive

methods that are not easily accessible in routine clinical practice.

Objective: To determine the anthropometric, clinical, and metabolic parameters that allow for the discrimination of type 2 diabetic patients who have insulin resistance from those who do not.

Methods: A cross-sectional analytical observational study was carried out in 92 type 2 diabetic patients. A discriminant analysis was applied using the SPSS statistical package to establish the characteristics that differentiate type 2 diabetic patients with insulin resistance from those without it.

Results: Most of the variables analyzed in this study have a statistically significant association with the HOMA-IR. However, only HDL-c, LDL-c, glycemia, BMI, and tobacco exposure time allow for the discrimination of type 2 diabetic patients who have insulin resistance from those who do not, considering the interaction between them. According to the absolute value of the structure matrix, the variable that contributes most to the discriminant model is HDL-c (−0.69).

Conclusion: The association between HDL-c, LDL-c, glycemia, BMI, and tobacco exposure time allows for the discrimination of type 2 diabetic patients who have insulin resistance from those who do not. This constitutes a simple model that can be used in routine clinical practice.

P-5

Short MCU variant forms plasma-membrane calcium pathway in the platelets

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Introduction: MCU, a pore forming subunit of mitochondrial Ca^{2+} ($mtCa^{2+}$) uniporter complex (mtCUC) regulates major $mtCa^{2+}$ influx pathway in various cell types/tissues. Human MCU gene is predicted to produce mRNA that coding a shorter transcript variant (termed MCU-S) in addition to canonical full length MCU transcript (renamed as MCU-L), but its functional relevance in human cells is not fully investigated.

Objective: MCU-S variant forms Ca^{2+}-permeable channels outside of mitochondria.

Methods: Human platelets from adult healthy donors and HEK293T cells stably overexpressing MCU-S were used for biomechanical, cell biological, and physiological assays.

Results: The existence of the mRNA and the protein of MCU-S was confirmed in various cells/tissues with the highest levels in platelets. MCU protein and several other mtCUC components were detected in plasma membrane of human platelets. Conventional whole-cell patch clamping in HEK293T cells stably expressing MCU-S exhibit an inward Ca^{2+} current sensitive to the conventional MCU blocker, Ru360. Human platelets also possess the Ru360-sensitive, but BTP2 (YM-58483, a selective inhibitor for the store operated Ca^{2+} entry [SOCE])-insensitive Ca^{2+} permeabilization. Human platelets showed Ru360-sensitive Ca^{2+} permeabilization via plasma membrane after switching the extracellular Ca^{2+} concentration from 0 to physiological range (~ 2 mM) in the presence of BTP2.

Conclusion: MCU-S variant is capable of forming Ca^{2+}-permeable channels outside of mitochondria, such as plasma membrane. Elucidation of the role of MCU transcript variants may provide us new insights into the molecular basis of mitochondrial and cellular Ca^{2+} handling in human platelet activation under physiological and pathological conditions.

Financing: NIH/NHLBI R01HL136757

P-6

Moderate and High-Intensity aerobic exercise exert differing metabolic benefits in candidates to undergo bariatric surgery. A randomised controlled trial

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Introduction: Exercise programs for candidates to undergo bariatric surgery are key for the preparation towards post-surgical fast-pacing weight loss. However, the optimal exercise prescription is unknown. Moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) are frequently prescribed, nevertheless, no clinical studies are known to compare them in this population.

Objective: To compare the metabolic effects of MICT and HIIT in candidates to undergo bariatric surgery.

Methods: Candidates to undergo bariatric surgery (gastric sleeve) were recruited and randomly allocated to MICT or HIIT. MICT was walking/cycling at 50% of heart rate reserve (HRR) for 30 minutes, whereas HIIT consisted of 6 bouts (walking/cycling) at 80% of HRR (2.5 min each) and 6 periods of active rest at 20% of HRR (2.5 min each). Both training programs consisted of 10 sessions performed during a 4-week period. Anthropometric measures, glycaemia, insulinaemia, HOMA-IR (both fasting and 120 minutes after oral glucose tolerance test (OGTT)), HbA1c, and circulatory fibroblast growth factor 21 (FGF21) were measured before and after the intervention.

Results: Participants after MICT (n=14) significantly reduced body weight, BMI, fat mass, and increased muscle mass. Also, reduced insulin levels after the OGTT ($p<0.05$). In contrast, HIIT (n=11) reduced HbA1c (Mean difference:-0.23 vs. -0.13) levels and FGF21 plasmatic concentrations ($p<0.05$). Both programs significantly reduced waist and hip circumferences (Mean difference:4.5vs7.7 cm, $p<0.05$).

Conclusion: Both MICT and HIIT exert metabolic benefits in candidates to undergo bariatric surgery in a specific manner. This might suggest that aerobic exercise intensity could target specific physical and metabolic dysfunctions in people with obesity.

Financing: National Agency of Research and Development (ANID) through an Early Career Research Grant (FONDECYT de iniciación en investigación), code 11200391

P-7

Circadian rhythms alterations during overwintering at the high-altitude Antarctic Station CONCORDIA (ESA/DLR CardiCorTEx Project)

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Introduction: Overwintering in Antarctica is considered as analogue for space missions, because humans are exposed to isolation, confinement, sleep deprivation and long-lasting darkness. The latter is known to trigger changes in the day-night rhythm (circadian) of physiological functions, leading to serious health and cognitive consequences, which might be relevant especially for astronauts.

Objective: The aim of this project is to assess and investigate circadian rhythm alterations during overwintering, including the additional effect of high-altitude (hypobaric hypoxia) on the cardiovascular system.

Methods: Participants (n=20) spend 12 months at the Antarctic station Concordia (3233 m a.s.l.), where “polar nights” (days without sunrise) occur during the winter and “polar days” (days without sunset) during the summer. During the whole overwintering physical activity is continuously recorded using actimetry. Furthermore, a 36-h monitoring (1/month) of core body temperature (CBT), heart rate, and nocturnal blood oxygen saturation is conducted (Ethic Approval EA4-132-19).

Results: First observations (n=10) show that the number of steps/day is significant lower during polar nights, increasing with the hours of sunlight/day. Moreover, as sunlight decreases, participants wake up later (linear mixed models, time course changes - $p<0.05$), which corresponds to changes in the lowest CBT value, occurring 1 hour later during polar nights.

Conclusion: CBT and actimetry data independently show dramatic changes of crew members circadian rhythm and behavior during polar nights, confirming our hypothesis. To preserve health and cognitive function countermeasures are needed, as for example

regular physical exercise. However, we will be able to draw conclusion only at the end of data collection.

Financing: European Space Agency (ESA) selected StudyGerman Aerospace Center (DLR) Grant Number 50WB2117

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P-8

Exploring the anion transport mechanism of the AE4 (SLC4A9) Cl-/cation-HCO3- exchanger

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Introduction: The Cl^-/cation-HCO_3^- exchanger AE4 plays a role in the Cl^--dependent fluid secretion in salivary glands. Although it is well established that AE4 transports HCO_3^- and Cl^-, it is not known whether other anions are also recognized as substrates.

Objective and methods: Here, we investigated the anion transport mechanism of AE4, using anion transport assays, site-directed mutagenesis, and molecular modeling. Results are shown as means ± SEM of 5 independent experiments, from at least 3 different transfections of human AE4 into the HEK-293 cell line. Student's t-test or one-way ANOVA followed by Bonferroni´s post hoc test was performed. A significant difference was considered with $p < 0.05$.

Results: We found that *wild-type* AE4 mediates anion/Cl^- exchange activity in the presence of bicarbonate, acetate, lactate, and oxalate. However, the Cl^- extrusion rates were lower for organic anions compared to HCO_3^-. Additionally, an EC_{50} of 8.7 mM was determined for HCO_3^- transport. As in other SLC4 transporters, the ion binding site in AE4 is at the TM3-TM10 interface, according to a homology model. We mutated 5 residues by aspartate in this region to investigate whether negative charges might affect anion transport. Mutants T448D and G449D, decreased HCO_3^- transport by ~40%, while A755D, T756D, and V757D, decreased transport by ~60, 70, and 40%, respectively. Moreover, our molecular simulations showed that negative charges at TM3-TM10 region modify electrostatic potential and promote structural rearrangements.

Conclusion: Our results indicate that AE4 transports organic anions, in addition to HCO_3^- and Cl^-, and the anion binding site is located at the TM3-TM10 interface.

Financing: Núcleo Milenio de Enfermedades Asociadas a Canales Iónicos (MiNICAD), Ministerio de Economía, Fomento y Turismo, Gobierno de Chile; Fondecyt # 11150454 (GPM), Fondecyt # 1191868 (SB), Fondecyt # 1191133 (WG).

P-9

Going through clots: deciphering the role of adrenergic stimulation in sepsis-induced disseminated intravascular coagulation in rats

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Introduction: Sepsis is the organ dysfunction promoted by the dysregulated response to an infection. The occurrence of coagulation abnormalities and its subsequent progression to disseminated intravascular coagulation (DIC) constitutes a significant risk for organ failure and mortality in septic patients. Elevated plasma catecholamine levels during sepsis are also correlated with unfavorable outcomes. Despite these findings, there is a lack of studies addressing the involvement of β-Adrenergic receptors (β-AR) in coagulation alterations during sepsis.

Objective: The aim is evaluating the contribution of β-AR in sepsis-induced DIC in rats.

Methods: Male SD rats aged 8 weeks were injected with bisoprolol or butaxamine at 5 mg/kg and 10 mg/kg respectively, or saline (n=3) and 1 h later, infused with endotoxin (LPS O55:B8, 30 mg/kg, 300 µL/h). After 3 h, blood and organs were collected for coagulation, biochemical and histological analysis. Additionally, EAhy.926 cells were pretreated with bisoprolol (1 µM), butaxamine (1 µM), endotoxin (20 µg/mL) or isoproterenol (1 µM) and co-cultured with platelets to evaluate adhesion. Results are presented as mean ±SD. Differences were assessed by two-ways ANOVA and Tukey's post-hoc test. Experimental protocols were approved by the Bioethics and Biosafety Committee of the Universidad Andrés Bello (approval number 037/2020).
Results: Endotoxin-treated rats exhibited altered DIC-related parameters and organ dysfunction. β_1-AR inhibition prevented these alterations. In ECs, the β_1-AR antagonist prevented platelet adhesion.
Conclusions: The β_1-AR stimulation modulate DIC-related parameters in endotoxemic rats and platelet adhesion in ECs. β_1-AR stimulation could be considered as target to reduce coagulation alterations and organ dysfunction septic patients.
Financing: Fondecyt Regular 1201039, Milennium Institute on immunology and inmunoterapy (ICN09_016/ICN2021_045: former P09/016-F), ANID scholarship 21220694.

P-10

Role of α-1 adrenergic receptor activation in platelet adhesion and coagulation protein expression in microvasculature HULEC 5-a cells under endotoxic condition

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Introduction: Sepsis syndrome is an uncontrolled inflammatory response to infection, with a high global incidence. During sepsis, there is an increase in circulating catecholamines, leading to the overactivation of α and β adrenergic receptors. This overactivation disrupts the hemostatic system, converting endothelium into a procoagulant phenotype, promoting endothelial cell activation, platelet adhesion and the expression of adhesion molecules such as P-selectin, von Willebrand Factor (vWF), and the αVb3 integrin, supporting thrombus formation and consolidation in the microvasculature.
Objective: To determine the role of α-1-adrenergic receptor activation in platelet adhesion to HULEC 5-a microvascular cells and the expression of the adhesion proteins vWF, P-selectin and αVb3.
Methods: HULEC5-a microvascular cells were pretreated with α-1 adrenergic agonist, phenylephrine and simultaneously stimulated with lipopolisacárido (LPS) or vehicle by 24 h, and platelet adhesion to EC was evaluated (n=3). Furthermore, immunofluorescence experiments were performed to evaluate the expression of adhesion proteins in EC (n=3). Results are represented as mean ± S.D. Significant differences were assessed by two way- ANOVA and Sidak´s post hoc test. Protocols were approved by institutional Bioethics Committee of the Universidad Andrés Bello resolution N° 002/2020.
Results: HULEC5-a microvascular cells pretreated with phenylephrine showed decreased platelet adhesion to EC exposed to LPS. In addition, decreased expression of P-selectin, vWF and αVb3 in EC exposed to LPS was observed.
Conclusion: α1- adrenergic receptor stimulation in HULEC5-a microvasculature cells attenuates the proadhesive phenotype.
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P-11

Gestational diabesity induced higher endothelium-derived hyperpolarization, associated with higher expression of beta 1 subunit of BKCa channels in HUVEC

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Introduction: The endothelium–derived hyperpolarization (EDH) mechanism could be altered in gestational diabesity (GD). EDH is a

vasodilation mechanism that depends, in part, of large conductance calcium–activated potassium channels (BKCa) expression. The EDH/BKCa mechanism has not been described in GD endothelium.

Objective: To determine the effects of GD on BKCa expression and EDH.

Methods: Umbilical cords from pregnant women with GD or healthy were collected (Ethic committee approval code CEC-SSC 21-04-24). HUVECs were isolated (collagenase 0.25 mg/mL, 37°C), growth in primary culture media and later incubated with insulin (1-10 nM). Immunofluorescence was performed using antibodies anti α and β1 BKCa subunits, analyzed in confocal microscope. The EDH was evaluated using $DiBAC_4(3)$ probe and quantified in the INCUCYTE system. Values are mean ± SEM and significant changes ($p<0.05$) were determined under Mann-Whitney's test.

Results: Immunofluorescence showed 2-fold increase of α and 3.5-fold increase of β1 subunits on GD-HUVECs. The β1:α relation showed no difference between GD and normal cells. Interestingly, the fluorescence distribution of β1 subunit was higher in apical plasma membrane in GD-HUVECs. Insulin Increases α but decreases β1 subunits expression in GD, decreasing 75% the β1:α relation. $DiBAC_4(3)$ fluorescence was 25% lower in GD, suggesting a higher EDH. Furthermore, insulin's effect on EDH was 82% reduced in GD compared to normal cells.

Conclusion: In GD, the higher expression and distribution of β1 subunit to the apical plasma membrane could be related to higher activation of BKCa, which could explain the increase of EDH in the pathology.

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P-12

Studying a Kir7.1 mutation associated with Leber´s congenital amaurosis (LCA)

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Introduction: Potassium channel Kir7.1 is highly expressed in retinal pigment epithelium (RPE). Human Kir7.1-mutations are associated with retinopathies like Leber's congenital amaurosis (LCA) evidencing a relationship between vision and Kir7.1 function. Here we show you the results obtained when studying the LCA-associated mutation Kir7.1-I120T.

Objective: Explore the Kir7.1-I120T properties and its mouse model effects.

Methods: Kir7.1-I120T function was studied in transfected HEK-293 cells or in RPE isolated from $Kir7.1^{I120T/WT}$ and $Kir7.1^{WT/WT}$ mice using patch-clamp technique. Kir7.1-I120T membrane expression was evaluated in biotinylation assays. Mouse vision was evaluated by electroretinography (ERG). Visual mice response was evaluated through behavioral assays (*Visual Looming* and *Visual Placing)*. Mouse experiments were approved by the institutional ethics committee (N°EP:2023-01-EP). Data is presented as mean ± SE (n>3) and evaluated with fisher's or t-test.

Results: HEK-293 cells expressing Kir7.1-I120T were evaluated through patch-clamp and biotinylation presenting no currents and normal membrane expression respectively. $Kir7.1^{I120T/I120T}$ mice die perinatally, such as Kir7.1-KO mice, but the $Kir7.1^{I120T/WT}$ mice shows normal development. $Kir7.1^{I120T/WT}$ RPE have Kir7.1 diminished currents (-2.5±0.5nA vs -6.1±0.4nA). $Kir7.1^{I120T/WT}$ and $Kir7.1^{WT/WT}$ mice presents indistinguishable ERG responses [A-wave (177.6±18.4μV vs 164.7±21.5μV), B-Wave (459±21.2μV vs 483±30.8μV) C-Wave (1324.2±64.9μV vs 1484.8±73.6μV)] and indistinguishable responses in behavioral assays (Fisher's test $p>0.240$).

Conclusions: Although Kir7.1-I120T exhibited a normal expression, it hasn't currents evidencing a non-functional channel. $Kir7.1^{I120T/I120T}$ exhibited a lethal phenotype likes Kir7.1-KO. $Kir7.1^{I120T/WT}$ RPE presents half of the current from WT animals, nevertheless these mice don't exhibit visual responses alterations in ERG or in behavioral assays. Reduced Kir7.1 activity in $Kir7.1^{I120T/WT}$ mice is sufficient to ensure normal development and visual function.

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AREA: CARDIOVASCULAR AND RESPIRATORY

P-13

Role of fibroblast growth factor 15 in myocardial function and hypertrophy in an experimental model of gallstone disease

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Introduction: Gallstone disease (GSD) is a multifactorial disease highly prevalent, and its cardiovascular consequences are still poorly understood. Previously, preclinical studies have shown increased circulating levels of fibroblast growth factor 15 (FGF15) in GSD. To date, no preclinical studies have investigated the mechanism underlying GSD with cardiovascular disease.

Objective: To evaluate whether FGF15 levels correlate with myocardial dysfunction and hypertrophy in an experimental model of GSD.

Methods: All experiments were conducted according to the Bioethics Committee of Universidad de Chile (CICUA-CQyF2021-34). Eight-week-old female and male C57/BL6N mice were assigned into two groups: Chow diet (Control; n=9) and lithogenic diet (GSD; n=7) to promote GSD. After 9 months, an echocardiography (ECO) was performed. After sacrifice, hearts and plasma were collected. We used an *in vitro* model of neonatal rat ventricular myocytes (NRVM) to study the heart-specific mechanism of FGF15. Mann-Whitney test was used for statistical analysis (Mean ± S.D.).

Results: GSD decreased ejection fraction (83.8 ± 4.7% vs 93.0 ± 1.2%, $p<0.001$), increased cardiomyocyte cross-sectional area (525 ± 55 vs 372 ± 101 µm, $p<0.05$), increased coronary artery thickness (14.0 ± 1.9 vs 11.5 ± 1.0 µm, $p<0.05$), increased cardiac β-MHC mRNA levels (4.7 ± 2.9 vs 1.2 ± 0.6, $p<0.05$) and tended to increase FGF15 serum levels (64 ± 29 vs 26 ± 7 ng/ml, $p=0.11$). Interestingly, FGF15-treated NRVM increased hypertrophy-related genes significantly.

Conclusion: GSD alters cardiac function and promotes cardiac hypertrophy. However, more research is required to analyze if FGF15 is involved in myocardial function during GSD development.

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P-14 ★*selected for oral communication*

Inhibition of mitochondrial protein kinase D protects right ventricles from cardiac fibrosis and dysfunction under pulmonary arterial hypertension

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Introduction: Activation of protein kinase D (PKD) in cardiomyocytes is a critical factor for pathological myocardial remodeling in response to left ventricular (LV) pressure overload. However, under right ventricular (RV) pressure overload such as pulmonary arterial hypertension (PAH), we previously showed that PKD activation occurs only in cardiac fibroblasts (CFs) in the RV, but not in cardiomyocytes, which is notably different from LV remodeling. Using RV-CFs isolated from Sugen5416/hypoxia (SuHx)-induced rat PAH model, we also reported the potential association of PKD activation at the outer mitochondrial membrane (OMM), increased mitochondrial reactive oxygen species, and RV-CF hyperproliferation.

Objective: CF-selective and mitochondria-targeted PKD inhibition *in vivo* attenuates RV fibrosis in PAH.

Methods: Adeno-associated virus serotype-9 (AAV9) carrying human TCF21 (hTCF21) promoter and OMM-targeted dominant-negative PKD (mt-PKD-DN) or its control AAV9-hTCF21-GFP/luciferase were injected in Sprague-Dawley rats via tail veins 10 days before Sugen5416 injection, followed by 3-week hypoxia and 4-week normoxia (protocol ID: 2105-39060A).

Results: Organ (i.e., heart) and cell-type (i.e., CFs)-specific GFP expression was confirmed in AAV9-hTCF21-GFP-injected rats. Both AAV9-hTCF21-mt-PKD-DN and -luciferase injections did not significantly alter baseline cardiac function.

However, AAV9-hTCF21-mt-PKD-DN significantly reduced RV hypertrophy and improved *in vivo* RV function after SuHx compared to AAV9-hTCF21-luciferase ($p < 0.05$, unpaired t-test). AAV9-hTCF21-mt-PKD-DN-injected SuHx rats showed a significant decrease in RV-CF proliferative signaling and RV fibrosis compared to AAV9-hTCF21-luciferase-injected SuHx rats ($p < 0.05$, unpaired t-test).

Conclusion: Mitochondrial PKD activation contributes to RV-CF hyperproliferation under PAH and may be a potential therapeutic target for PAH-induced RV fibrosis.

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P-15

Competing endogenous long non-coding RNAs in heart failure as potential regulators of colon cancer genes

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Introduction: Cardio-oncology field has been studied mostly from a cancer view as the cause of cardiac dysfunction. Lately, epidemiology and pre-clinical reports revealed heart failure (HF) affects cancer progression by a mechanism related to circulation factors. Newly long non-coding RNAs (lncRNAs) are mainly reported as HF biomarkers, but transcriptomic analysis has shown an important number of lncRNAs with unknown pathophysiological relevance yet. Besides, lncRNAs, also known as competing endogenous RNAs (ceRNAs), regulate gene expression, a mechanism described in cancer and HF.

Objective: To investigate ceRNAs in HF as cancer progression regulators**.**

Methods: Differentially expressed genes (DEG) analysis of public RNA-seq datasets of HF left ventricle and blood, and colon cancer tumor samples (≥ 4) were assessed to obtain cancer target mRNAs (Log2FC > 1,5, p-value adj < 0,05) and HF lncRNAs (Log2FC > 0,5, p-value adj < 0,05). DEG lncRNA tissue characterization was assessed by reanalyzing available data from the GTEx portal and Tau tissue-specificity index metric. ceRNA networks were identified by cross-referencing lncRNAs candidates with an in-house developed human interaction network built based on experimentally validated lncRNAs-miRNAs and miRNAs-lncRNAs interactions. Finally, cancer mRNAs targets gene ontology enrichment analysis was assessed.

Results: We identified 16 lncRNAs DEG in common between HF left ventricle and blood. Among them, 6 ceRNAs were predicted to target miRNAs, which regulate genes related to mitotic cell cycle and epithelial-mesenchymal transition processes.

Conclusion: HF patients' blood lncRNAs could regulate colon cancer progression genes through a ceRNA mechanism. Further *in vitro* assays are needed to the objective validation.

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P-16

Cardiac autonomic modulation during sleep in miners exposed to chronic intermittent hypoxia in Chile

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Introduction: Research on cardiovascular responses to the chronic intermittent hypoxia (CIH) model in Chile, especially during nocturnal sleep in hypertensive miners under real working conditions, has been limited.

Objective and Methods: This study aimed to investigate the cardiac autonomic responses during nocturnal sleep in miners exposed to CIH for over two years, including hypertensive (n=10) and non-hypertensive (n=10) workers.
Participants aged 47±7 years, were overweight (BMI 29.2± 3) and worked on 7-on 7-off days shift between high altitude (HA: >4,200 masl) and sea level (SL: <500 masl). The study received ethical approval from the University of Antofagasta's Ethics Committee (number: 181/2019). Data were recorded by 1-lead electrocardiography (ECG) during a window of 4-hour sleep during the first night at HA and after the second night at SL. A two-way ANOVA was employed to analyze interactions between two independent variables.
Results and Conclusion: Compared to SL parasympathetic indices of HRV were lower in both groups at HA, either in time domain (RMSSD 36.89±15.5 ms SL vs. 25.91±15.0 ms HA; p <0.05) and in frequency domain (log HF 5.90±0.9 ms^2 SL vs. 4.71±0.1 ms^2 HA; p<0.05), with a group x altitude interaction for RMSSD (p=0.02) and a tendency for log HF (p=0.06). Non-linear HRV domain supported a decrease of vagal tone at HA (DFA1 1.08±0.2 SL vs. 1.59± 0.2 HA; p<0,01) and indicated reduced signal complexity (SampEn 1.61±0.1 SL vs.1.15±0.13 HA; p<0.01). Despite ongoing treatment, prolonged CIH exposure did not prevent acute cardiovascular changes at HA, with a more pronounced decrease in vagal tone observed in hypertensive workers.
Financing: This is investigation was supported by ANID/ FONDECYT INICIACIÓN / 11180503/ (Chile).
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P-17

The effect of mechanical stress on the primary cilium in vascular smooth muscle cells

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Introduction: Vascular smooth muscle cells (VSMCs) present a primary cilium physiologically. This organelle receives and transduces mechanical signals and is highly relevant in the development of pathologies. Given the association between these structures in VSMCs and the response to signals of mechanical origin, such as physiological compression stress or hypertension, it is relevant to study the effects of this signal on the organelle.
Objective: To study the effect of mechanical stress on the primary cilia of VSMC, both in cell lines and tissues.
Methods: Mechanical stretch was induced by hypoosmotic (HS) solution in A7r5 cell line and hypertensive male C57BL/6-mice equipped with osmotic mini pumps delivering AngII (FOUCH#130806) were used. Primary cilium was counted by immunocytochemistry (ICC) against acetylated tubulin (n=4). Cell areas were measured by ICC against α-SMA (n=3), and IFT88 protein levels were evaluated by Western blot (n=3). Remodeling and thickness of the VSMCs layer in aortic rings were measured by hematoxylin/eosin and Masson staining (n=3). Statistical analyses were performed by T-test, and a p< 0.05 (mean ± S.E.M) was determined as a significant difference.
Results: HS induced loss of primary cilium but IFT88 levels do not change upon application of HS in A7r5 cells. Increased presence of collagen and thickness was observed in aortic rings obtained from hypertensive, associated with a tendency to decrease the primary cilium in VSMCs.
Conclusion: Mechanical stress induces disassembly and loss of primary cilium in VSMCs and therefore could be involved in the context of the development of cardiovascular pathologies.
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P-18

B-cell lymphoma protein 6 (BCL6): a novel transcription factor involved in mitochondrial dysfunction-induced cardiac hypertrophy

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Introduction: Heart failure (HF) is a condition where the heart cannot pump enough blood to the body. HF onset is preceded by cardiac hypertrophy (CH), an adaptative response characterized by cardiomyocyte enlargement. Cardiac stressors, such as Norepinephrine (NE), activate a global transcriptional response of master transcription factors (TFs, e.g., NFAT, GATA4, MEF2), however, these already known TFs do not account for all the changes observed during the CH process.

Objective: To identify novel TFs involved in HF/CH.

Methods: Using a systems biology approach, we build a transcriptional regulatory network (TRN) activated by HF. The TRN contained uncharacterized factors in CH/HF, thus selecting BCL6 as a potential new regulator. To evaluate BCL6 function, we treated neonatal rat cardiomyocytes (NRVM) with NE. All procedures were approved by CICUA committee (22538-CYQ-UCH). Data were analyzed by unpaired t-test or ANOVA; values corresponded to mean ± SEM (N = 3-5).

Results: BCL6 mRNA and protein levels increased on NE-treated NVRM. BCL6 expression and nuclear distribution correlated with the overexpression of cardiomyocyte hypertrophy markers (ANP and BNP) and cell growth. In addition, BCL6 knockdown (KD) on NE-treated NVRM ameliorated ANP and BNP increase and inhibited cardiomyocyte hypertrophic growth. Furthermore, BCL6 KD reverted Drp1 phosphorylation-induced mitochondrial fragmented phenotype and upregulated Mitofusin-2 (Mfn2) levels, both characteristics of the metabolic switch observed in CH.

Conclusion: Our results suggest that BCL6 may exert a critical pro-hypertrophic role in CH regulating mitochondrial dynamics. Further studies will be needed to BCL6 mitochondrial function and its potential therapeutic role in HF.

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P-19

Miro1 is necessary for fructose-induced cardiomyocyte hypertrophy

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Introduction: Understanding the impact of consuming added sugars is highly relevant. Sweeteners such as fructose represent a major source of carbohydrates in the modern diet. Fructose consumption has been associated with cardiovascular diseases such as hypertension and cardiac hypertrophy (CH). Mitochondria are responsible for 90% of ATP production in cardiomyocytes and changes in mitochondrial function play a central role in CH. Miro1 is a mitochondrial outer membrane protein involved in mitochondrial axonal movement in neurons. Although Miro1 is expressed in the heart, little is known regarding its role in fructose induced cardiomyocyte hypertrophy.

Objective: To evaluate whether Miro1 is necessary for cardiomyocyte hypertrophy induced by fructose.

Methods: We used neonatal rat cardiomyocyte cultures (CBA#1246-FMUCH) treated with fructose (25 mM, 48 h). To generate Miro1 knockdown, cardiomyocytes were transfected with a siRNA specific to Miro1 (siMiro). To determine cardiomyocytes hypertrophy after fructose treatment (control and siMiro cardiomyocytes) we evaluated 1.- cellular area by epifluorescence microscopy 2.- mRNA levels of ANP, BNP and β-MHC and mitochondrial content by qRT-PCR 3.- β-MHC protein levels by western blot. Data were shown as mean ± SEM of 5-7 independent experiments. Student's unpaired t-test or two-way ANOVA were used to compare means among 2 or ≥3 groups respectively.
Results: Fructose increased cellular area, β-MHC protein levels, mRNA levels of ANP, BNP and β-MHC hypertrophic markers together with a decrease in mitochondrial content. Miro1 knockdown avoided cardiomyocyte hypertrophy induced by fructose.
Conclusion: Miro1 is necessary for fructose-induced cardiomyocyte hypertrophy.
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P-20

Concurrent, dual perturbations of the 5-HT and NA systems reveal dynamic interplay in regulating the neonate autoresuscitation reflex

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Introduction: Sudden Infant Death Syndrome (SIDS) is a leading cause of infant mortality worldwide. Postmortem studies indicate abnormalities in the serotonergic (5-HT) and noradrenergic (NA) systems of the respiratory network.
Objective: To better understand the singular and compounded contributions of these two neurotransmitter systems to SIDS pathology by interrogating their role in the autoresuscitation reflex.
Methods: I developed a novel series of conditionally expressed inhibitory and excitatory designer receptors activated by designer drugs (DREADD) models that allows for singular excitation or inhibition of either the whole 5-HT or whole NA systems as defined by cre- and FLPo-drivers, respectively. These models were then uniquely combined to further enable 1) concurrent stimulation or inhibition of both the NA and 5-HT systems in the same animal or 2) inhibition of one system while exciting the other system in the same animal. Mice were repeatedly assayed for the autoresuscitation reflex using our new closed loop robotic neonate cardiorespiratory assessment platform. All statistics result from a linear mixed effects model with a Tukey HSD post-hoc.
Results: In 320 mice, I found that NA ($p=5.11x10^{-8}$, n=47) and 5-HT (p=0.002, n=26) activation alone significantly decreased survival compared to controls (n=65). When NA activation was combined with 5-HT activation ($p=3.12x10^{-12}$, n=27) or inhibition ($p=6.37x10^{-6}$, n=19), the reduction in survival persisted. However, when NA inhibition was combined with 5-HT activation (p=0.052, n=28) or inhibition (p=0.95, n=33) there was no change in survival.
Conclusion: NA modulation overpowers effects of 5-HT modulation and rescues effects of 5-HT activation when combined.
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P-21

Effects of 17β-estradiol and its metabolite 4-Methoxyestradiol in the proliferation and mitochondrial dysfunction of human pulmonary arterial smooth muscle cells

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Introduction: Pulmonary arterial hypertension is an arteriopathy more prevalent in women, which has been associated with high levels of 17β-estradiol. This disease is characterized by a cancer-like metabolic shift, increased Reactive Oxygen Species (ROS) production and a proliferative phenotype of primary human pulmonary arterial smooth muscle cells (PASMC) that leads to vascular remodeling, but whether 17β-estradiol or any of its metabolites, like 4-Methoxyestradiol,

can directly trigger a pathological phenotype in these cells is unknown.

Objective: To evaluate the effect of 17β-estradiol and 4-Methoxyestradiol in proliferation, ROS production, and metabolism of PASMC.

Methods: We treated human PASMC with 17β-estradiol (100 nM) and 4-Methoxyestradiol (10 nM) for 48 h and proliferation was determined by MTS assay and Trypan blue. Oxygen consumption rate (OCR) and mitochondrial membrane potential (Δψm) were evaluated using Oroboros.

Mitochondrial ROS (mtROS) were assessed by Flow Cytometry. Protein and mRNA levels of peroxiredoxins (1-6) and NOX4 were determined by Western blot and rt-qPCR, respectively. Data were analyzed using Kruskal-Wallis test and were expressed as mean ±SEM.

Results: Both estrogens increased proliferation (46.8% ± 8 for 17β-estradiol and 42.2% ± 5.6 for 4-Methoxyestradiol, n=5), mtROS (105.2% ± 39.5 for 17β-estradiol and 167.1% ± 82.1 for 4-Methoxyestradiol, n=4) and OCR (17β-estradiol basal OCR and 4-Methoxyestradiol state 3 OCR, n=4). Both treatments reduced Δψm and preliminarily increased NOX4 protein levels (n=2) but had no effect on its mRNA. Only 4-Methoxyestradiol increased mRNA levels of peroxiredoxins.

Conclusion: Both estrogens promote cancer-like phenotype in hPASMC, but 4-Methoxyestradiol may have a differential effect on cellular redox balance.

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P-22

Asthma enhances systolic blood pressure increase along infancy: The AsmaVix Project

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Introduction and Objective: The aim was to investigate the influence of asthma on age-dependent systolic blood pressure (SB) increase in children.

Methods: Asthmatic (Asth, n=166) and non-asthmatic (N-Asth, n = 50) children (7-15 years) attending to Basic Health Units of Vitória were included. Clinic and laboratory exams were performed in an outpatients' clinic at the University Hospital. Blood Pressure (BP) was measured under standard conditions (oscillometric device, Onrom HBP 1100). The 90th percentile SBP or DBP was used to identify high BP considering gender, age and height. Data are means ± sd of 95% confidence limits (CL). Statistical analyses were performed using the student t-test and Pearson's regression analysis. Significance was set at $p<0.05$. The project was approved by the institutional ethics committee (CAAE 09214519.1.0000.5071).

Results: Asth and N-Asth groups were similar ($p>0.05$) for age (11.7 ± 2.3 *vs* 11.1 ± 2.1 years), body mass index (20.2 ± 4.2 *vs* 20.3 ± 5.7 kg/m^2), SBP (102 ± 11 *vs* 104 ± 10 mmHg) and DBP (62 ± 7 *vs* 63 ±8 mmHg). The crude age-dependent SBP increase was 34% higher in the Asth group (1.74 mmHg/year; 95%CL 1.06;2.43 *vs* 1.37 mmHg/year; 95% CL 0.02;2.72). The difference between groups remained after adjusting for gender and body mass index (1.47 mmHg/year; 95%CL 0.80; 2.14 vs 1.10; -0.47;2.67). Percentage of children with elevated BP was twice higher in the Asth group (10.2% vs 4.0%).

Conclusion: Presence of asthma accelerates the SBP increase along childhood and may predispose to an earlier onset of hypertension.

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P-23

Development of a chronic cardiotoxicity model induced by Doxorubicin in female C57BL/6j mice

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Introduction: Breast cancer is the most prevalent type in women. Doxorubicin (DOX) is an efficient and widely used medication for it, however, depending of cumulative dose concentrations of treatment, up to 46% of the patients can present cardiotoxicity associated to cardiac atrophy, being the main limitation for its use. Until now preclinical models are predominantly based on male animals, without considerer that female sex is a risk factor for cardiotoxicity.

Objective: To standardize a preclinical female model of cardiotoxicity induced by doxorubicin treatment.

Methods: Female C57BL/6j mice (10-12 week old, FMUCH#21479) were weekly treated with intraperitoneal injections until achieving 15-40 mg/Kg cumulative dose of DOX and were subsequently followed for 5-11 weeks. Cardiac function was measured by echocardiography and heart weight/tibia length was obtained. Cardiac β-MHC, BNP and MAFbx/Atrogin1 were measured by qRT-PCR. Student's t test or one-way ANOVA and Tukey's post-test were used for statistical analysis and data were expressed as the mean ± SD (n=8).

Results: 30 mg/Kg dose of DOX in mice induced cardiotoxicity signs and 50% premature death. Heart weight decreased in DOX group (5.46 ± 0.66 mg/mm), premature death (4.83 ±0.54 mg/mm) and survivor to DOX sub-groups (5.91± 0.18 mg/mm) against to the control group (6.85 ± 0.58 mg/mm). Ejection fraction and mRNA markers did not change between mice with premature death or survivors to DOX.

Conclusion: Female mice model at 30 mg/Kg DOX dose develops the clinically relevant characteristics as well as the different cardiotoxicity sub-groups like in humans.

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P-24

Bridging the gap: The first rat model of sepsis-induced coagulopathy replicating key clinical features of patients

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Introduction: Sepsis is defined as a potentially life-threatening organ dysfunction caused by a dysregulated response to infection. During sepsis, a subclinical condition called sepsis-induced coagulopathy (SIC) appears, and in a more advanced stage, this is transformed into disseminated intravascular coagulation (DIC), a syndrome characterized by intravascular activation of coagulation without specific localization. This generates dysfunction of the microcirculation, causing organ failure, and ultimately promoting death. Both SIC and DIC are scores used to determine the progression and severity of septic patients.However, there is currently no animal septic model focused on categorizing the severity of sepsis by clinical scores.

Objective: To generate a highly representative and affordable rat model of SIC.

Methods: Eight-week-old male Sprague-Dawley rats were subjected to intravenous infusion of LPS (15 mg/kg/h) (n=4-8) or saline (n=4-8). At 3 and 23 h post-infusion, blood was collected and the following parameters were determined for SIC: INR, SOFA and platelet count. For DIC: plasma fibrinogen, prothrombin time (PT), platelet count and D-dimer. Results are presented as mean ± SD. Significant differences were assessed by two-way ANOVA and Šídák's post hoc test. Experimental protocols were approved by the Bioethics and Biosafety Committee of Universidad Andrés Bello.

Results: Endotoxemic rats showed a 2-fold increase in INR, 1.5 times PT and 100 times plasma D-Dimer. While platelet count and fibrinogen decreased by half. In addition, in SOFA we found that PaO2/FiO2 decreased by half, however there was no change in bilirubin and cratinine.

Conclusion: The model successfully replicates the key features observed in human patients with SIC.

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P-25

Improvement of the cardiovascular effect of methyldopa by complexation with Zn(II): synthesis, characterization and mechanism of action

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Introduction: α-methyldopa (MD) is used for managing hypertension during pregnancy. Zinc deprivation has been associated with many diseases.

Objective: The synthesis of a Zn coordination complex $[Zn(MD)(OH)(H_2O)_2].H_2O$ (ZnMD) provide a promising alternative pathway to improve the biological properties of MD.

Methods: Fluorescence spectral studies were conducted to examine the binding of complex with bovine serum albumin (BSA). MD, ZnMD, and $ZnCl_2$ were administered to spontaneous hypertensive rats (SHR) rats during 8 weeks and systolic blood pressure (SBP) and echocardiographic parameters were determined (CICUAL Protocol number: T02-02-2023). *Ex vivo* assays were conducted to evaluate oxidative stress, cross-sectional area (CSA) and collagen levels of cardiac tissues. The expression of NAD(P)H oxidase subunits ($gp91^{phox}$ and $p47^{phox}$) and Superoxide Dismutase 1 (SOD1) was quantified through western blot analysis. Values are mean ± SEM.

Results: MDZn ZnMD exhibited a moderate affinity for binding with BSA, involving Van der Waals forces and hydrogen bonds. Upon treatment in SHR, a reduction in SBP was observed, being ZnMD more effective than MD (122±8.1 mmHg and 145±5.6 mmHg, at 8^{th} week, respectively, n=4). ZnMD prevented myocardial hypertrophy, improved heart function and reduced cardiac fibrosis. In contrast, MD did not show noticeable differences in these parameters. ZnMD regulated negatively the oxidative damage and increased SOD1 expression, while MD did not show significant effect.

Conclusion: Both MD and ZnMD have the potential to be transported by albumin. Our findings provide important evidence suggesting that this complex could be a potential therapeutic drug for the treatment of hypertension and cardiac hypertrophy and dysfunction.

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P-26

Senescence of vascular smooth muscle cells: Effect of palmitate and Nω-nitro-L-arginine methyl ester (L-NAME)

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Introduction: Heart failure with preserved ejection fraction (HFpEF) is an age-related disease. It has been proposed that one of the main characteristics of HFpEF is vascular dysfunction. We propose that vascular smooth muscle cells (VSMCs) senescence plays a significant role in HFpEF-associated vascular dysfunction. HFpEF mouse model involves inducing nitrosative stress in the heart through a combination of a high fat diet and L-NAME. Therefore, we aimed to evaluate whether the treatment of A7r5 VSMC cultures with palmitate and L-NAME, stimuli that induce HFpEF *in vivo*, promotes cell senescence.

Objective: To determine whether VSMCs treated with palmitate and L-NAME, stimuli known to induce HFpEF in mice, triggers cell senescence.

Methods: Toxicity levels of L-NAME and palmitate were determined using dose-response assays, assessing cell viability through cell counting, MTT assays, and PI permeability via flow cytometry (n=4). Senescence was evaluated by assessing senescence-associated β-galactosidase activity (n=4), IL-6 release into the cell culture media by ELISA (n=7), and p53 expression through immunofluorescence (n=3). Data were analyzed using one-way ANOVA followed by post-hoc Kruskal-Wallis tests. Results are presented as the mean ± S.E.M.
Results: Non-toxic concentrations of L-NAME and palmitate, following 72 hours of stimulation, were 5 mM and 1 nM, respectively. These treatments changed A7r5 VSMC morphology and increased senescence-associated β-gal activity, IL-6 and p53.
Conclusion: The model generated by the treatment of A7r5 VSMC with 1 nM palmitate and 5 mM L-NAME for 72 hours triggers a senescent-like phenotype.
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P-27

Diaphragm weakness in germ-free mice is sex dependent

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Introduction: There is a growing recognition of the importance of gut-derived metabolites for the development, maintenance, and functional capacity of skeletal muscle.
Objective: In conventional (control) and germ-free male and female mice we assessed ex vivo force-generating capacity of diaphragm and extensor digitorum longus (EDL) muscles.
Methods: University College Cork Animal Welfare Body approved the study. Diaphragm and EDL muscles were removed from 6–10-month-old conventional C57 (17 male/10 female) and GF (12 male/14 female) mice for structural, functional, and molecular analyses.
Results: Diaphragm and EDL force was significantly lower in GF male mice compared with conventional mice. Forces were equivalent in female GF and conventional mice. Gene expression of receptors for microbially-derived metabolites (short chain fatty acids and secondary bile acids) were down regulated in GF males but not GF females. Two-way ANOVA with correction for multiple comparisons (Sidak) was used for all statistical comparisons.
Conclusion: Our data suggests that the absence of microbes is deleterious to skeletal muscle function in a sex-dependent manner. Further molecular analysis is ongoing to elucidate the key differences between sexes.
Financing: Funded by Science Foundation Ireland SFI/19/6628 INSPIRE DMD and the Department of Physiology, UCC.

P-28

Chronic N-acetyl cysteine treatment does not affect diaphragm fibrosis, immune cell infiltration or respiratory performance in the mdx mouse model of Duchenne muscular dystrophy

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Introduction: Duchenne muscular dystrophy (DMD) is characterised by respiratory muscle injury, leading to inflammation, muscle weakness, and fibrosis, ultimately leading to respiratory failure. The dystrophin-deficient mouse model of DMD (*mdx*) shows evidence of impaired respiratory muscle performance, fibrosis, and inflammation in early disease. N-acetylcysteine (NAC) has both anti-inflammatory and anti-fibrotic effects leading to improved muscle performance in a range of animal models of muscle dysfunction. The effects of chronic NAC administration on respiratory system performance in *mdx* mice was assessed.
Methods: One-month-old male *mdx* mice were randomised to receive normal drinking water (n=30) or 1% NAC in drinking water (n=30) for 3 months. At 4 months of age, we assessed breathing in conscious mice by plethysmography and ex vivo assessment of diaphragm force-generating capacity. Diaphragm samples were taken for structural analysis. In separate anaesthetised mice, respiratory electromyogram (EMG) activities and inspiratory pressure across a range of ventilatory behaviours was determined, including assessment of peak respiratory system performance. Two-way ANOVA and unpaired t-

tests were performed. Study approved by UCC ethics committee (AEEC 2019/013).
Results: NAC administration did not affect breathing or force-generating capacity of the diaphragm in *mdx* mice. Furthermore, there was no significant effect of NAC on inspiratory EMG activities or inspiratory pressure across behaviours, from basal to peak system performance. Collagen content, immune cell infiltration, and central nucleation were unchanged in *mdx* + NAC compared with *mdx* diaphragms.
Conclusion: We conclude that chronic NAC supplementation has no overt beneficial effects on respiratory system performance in the *mdx* mouse model of DMD.
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P-29

Hydrogen sulfide treatment attenuates cardiac hypertrophy and increases SERCA2a activity in hypertensive rats

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Introduction: Hydrogen sulfide (H_2S) protects the heart against hypertension-induced hypertrophy through mechanisms that have not been completely clarified. Under physiological conditions, H_2S regulates heart function by affecting the activity of proteins involved in excitation-contraction coupling, such as reticulum sarcoplasmic Ca^{2+} ATPase (SERCA2a). Nonetheless, it remains unknown whether the cardioprotective action of H_2S during hypertension is related to alterations in SERCA2a function.
Objective: In this work, we evaluated the effects of chronic administration of NaHS (H_2S donator) on the expression and activity of SERCA2a in rats made hypertensive by abdominal aortic coarctation (AAC).
Methods: 24 male Wistar rats were divided into four main groups: Sham (n= 6); AAC (n= 6); AAC + 3.1 mg/kg NaHS (n= 6); and AAC + 5.6 mg/kg NaHS (n= 6). The protocol was approved by Cicual-Cinvestav (374-07). Cardiac hypertrophy (CH) was assessed by the hypertrophic index and the expression of ANP and pERK. SERCA2a function was evaluated by determining the expression of SERCA2a and PLB, and by measuring the time constant (τ) during the decay phase of Ca^{2+} transients (CaT) induced by electrical stimulation in isolated cardiomyocytes. Data were presented as mean ± S.E.M and were evaluated using one-way ANOVA followed by a Tukey posthoc test.
Results: Administration of 5.6 mg/kg NaHS attenuated ACC-induced CH and decreased τ compared to Sham and AAC groups, but it did not alter the expression of SERCA2a and PLB.
Conclusion: These results suggest that cardioprotective role of H_2S during hypertension is accompanied by an increase in SERCA2a activity.
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P-30

Senescence in rat aorta vascular smooth muscle cells induced by iNOS

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Introduction: In reactive oxygen species (ROS)-mediated senescence, there is an accumulation of damaged molecules that cause oxidative stress, increasing the incidence of cardiovascular diseases. Chronic exposure to NO produced by iNOS during inflammation has been shown to induce senescence in several immortalized cell lines. However, there is no evidence linking iNOS directly to the generation of senescence in vascular smooth muscle cells (VSMCs).
Objective: To evaluate the effect of iNOS overexpression on senescence in rat aorta VSMCs.
Methods: HEK293 cells were used to amplify adeno-Lac Z and adeno-iNOS, and they were purified by a discontinuous CsCl gradient. A7r5 cells, passages 5-12, were transduced or not with adeno-Lac Z (control) or adeno-iNOS, using different multiplicities of infection (MOI) for 72 hours. They were then evaluated by western blot. The cytotoxicity of overexpression was evaluated by measuring cleaved Caspase-3 (not detected;

n=2). Senescence was assessed by p16 (0.77 and 1.45 fold change from control; n=2) and p21 by western blot (1.63 and 1.19 fold change from control; n=2), and γ-H2A.X by inmunofluorescence (1.18 fold change of Corrected Total Cell Fluorescence (CTCF) Integrated Density from control; n=1), at 3 days post-transduction.

Results: Overexpression of iNOS did not induce apoptosis, but increased p16, p21 and γH2A.X levels in VSMCs at 3 days post-transduction.

Conclusions: Our results suggest that iNOS induces senescence in VSMCs *in vitro*. However, further work is required to confirm these preliminary results.

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P-31

Development of imaging tools for evaluation of cardiac fibrosis

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Introduction: Cardiac fibrosis (CF) is a pathophysiological process characterized by an excess of extracellular matrix (ECM) deposition by cardiac fibroblasts (CFs). CF develops in myocardial infarction, hypertension, and different types of cardiomyopathies. There is no sensitive method for its diagnosis at an early stage of CF development Fibroblast activation protein (FAP) is an interesting protein found in activated CFs. Our project is oriented to synthesizing a new nanosystem that recognizes FAP.

Objective: To standardize an *in vitro* model of cardiac fibrogenesis induced by TGF-β1 to test new nanosystem recognizing FAP.

Methods: Primary cultured rat CBF were treated with TGF-β1 5 and 10 ng/ml at 24, 48, and 72 h. The presence of stress fibers in activated CFs was detected by collagen contraction gel assay, while α-SMA expression was assessed by indirect immunofluorescence. Protein levels of α-SMA, fibronectin, and collagen-I were determined by Western blot. One-way ANOVA, post hoc Tukey, *p< 0.05, mean ± SD (n = 3-6). CICUA-CQyF2023-50 code.

Results: TGF-β1 (10 ng/ml at 72 h) increased the contractile capacity of CFs. TGF-β1 10 ng/ml promoted α-SMA gene transcription and significant increases in cell área (characteristic of cardiac myofibroblast contractile phenotype), expression of Collagen type I, fibronectin, and α-SMA proteins.

Conclusion: TGF-β1 10 ng/ml induced differentiation to cardiac myofibroblast at 72h of stimulus. This model is being used to evaluate the new nanosystem designed to recognize FAP.

Financing: FONDECYT 1211482, FONDAP 15130011, Anillo 210068

P-32

Association between nutritional status, visceral fat level and alterations in blood pressure in undergraduate students from Valparaiso

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Introduction: The prevalence of hypertension in adolescents is increasing worldwide. Recent reports showed that elevated blood pressure (BP) in childhood and adolescence could lead to hypertension in adulthood. High body mass index (BMI) is strongly associated with elevated BP. BMI is a general indicator for obesity, but it cannot distinguish between body fat distribution, lean body mass (LBM) or visceral fat levels (VFL).

Objective: the aim of this study was to assess the associations of body composition with BP in normal-and overweight undergraduate students.

Methods: Body composition of 150 students from the Pontificia Universidad Católica de Valparaíso (PUCV) aged 19-22 years-old, was determined by using body impedance. Weight and height were measured. Systolic and diastolic BP was measured at rest using Omron HEM digital arm sphygmomanometer. The parametric and nonparametric statistical analyzes were carried out using the Graph Pad Prism6.0 program considering a confidence level of 95%. This study was approved by the ethical committee of the PUCV (BIOEPUCV-H-205-2018).

Results: Significant increases in Systolic BP were found in obese students compared to the normal weight group. Significant increase in Systolic BP was found in students with high VFL compared to those with normal VFL. A positive linear correlation ($p<0.05$) was observed between VFL and the average systolic BP. Finally, statistically significant associations were found between hypertension and VFL, as well as between nutritional status and hypertension.
Conclusion: Our results indicate that young students present VFL that impacts on arterial BP, then, this data may help for future public health decisions.
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Acute lead poisoning causes heart failure by direct action on the heart
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Introduction: Lead (Pb2+) is a heavy metal, that can cause acute or chronic poisoning without threshold dose. The typical form found in nature is similar compared with calcium (Ca2+).
Objective: We aimed to study if Pb2+ exposure can interfere with voltage gated Ca2+ channels, and intracellular Ca2+ management models, having the heart as a model, given its high dependence to Ca2+ for its proper functioning.
Methods and Results: The protocol had ethical approval by the Honorary Commission for Animal Experimentation (n°071140-001788-09). Paired-samples-T-test mean +/-SEM6. Guinea pig hearts isolated were retroperfused though the coronary arteries with Tyrode 1.8mM Ca2+. In those hearts, tension and electric response were registered simultaneously. Through optical fibers, these hearts were observed from within, exciting specific Pb2+ fluorophores (LG). Pb2+ entry was detected by LG staining, in almost all cells. A negative inotropic effect of Pb2+ and a prolongation of the duration of the compound action potential was observed in isolated hearts. Ventricular cardiomyocytes were isolated and were loaded with LG or Ca2+ sensitive pigments, to be later observed. The electric activity in isolated cells was recorded with the patch-clamp method. Cav1.2 currents were reversibly blocked by Pb2+. An increase in fluorescence of LG was observed. This increment was blocked with 1-5µM of Nifedipine suggesting that Pb2+ does not only block Cav1.2 channels but that also enters to the cardiomyocytes through them. The release and reuptake of Ca2+ were altered in the presence of extracellular Pb2+.
Conclusion: Pb2+ can alter cardiac function directly, entering cardiomyocytes through Cav1.2 channels.
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P-34 ★*selected for oral communication*
Obligatory and accessory muscle contribution to peak inspiratory performance in age-related sarcopenia
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Introduction: Respiratory pathology is implicated in age-associated disease and respiratory failure represents a distinct cause of mortality in older patients. This highlights the importance of understanding the impact of age-related muscle wasting on the respiratory phenotype. Data on the neural control of breathing in sarcopenia is scarce. Furthermore, accessory respiratory muscles have received little attention.
Objective: We aim to comprehensively characterise respiratory muscle activity of young mice using electromyography (EMG) combined with inspiratory pressure. Our objective is to employ this approach to investigate the impact of sarcopenia on the neuromuscular control of breathing.
Methods: Project authorisation was obtained from the Health Products Regulatory Authority (AE19130/P157). Male 6-month-old wild-type mice were studied (C57/BL10, *n*=7). Needle electrodes were inserted for contemporaneous measurement of EMG activity in diaphragm, external-intercostal, parasternal, scalene, cleidomastoid, sternomastoid, sternohyoid, and trapezius muscles. Respiratory pressures were measured using a pressure-tip catheter in the

thoracic oesophagus. Activity associated with peak pressure was recorded during sustained tracheal occlusion. Subsequently, activities were recorded following bilateral vagotomy, and in response to chemostimulation (F_{iO2}=0.15/F_{iCo2}=0.06). One-way paired t-test was performed using Prism (v10.1.0). **Results:** Peak pressure increased significantly from baseline to obstruction (-33.56 ± 5.73; mean ± SD). Associated diaphragm EMG activity increased 300%. Quiescent accessory muscles became highly active, with increases in cleidomastoid, sternomastoid, scalene, and trapezius.
Conclusion: Measurement of respiratory muscle EMG activity provides further insight into respiratory pressure generation during ventilatory and non-ventilatory behaviours. Employment of this protocol in older animals will enhance understanding of the impact of sarcopenia on neural control of the respiratory musculature.
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AREA: ENDOCRINE AND METABOLISM

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Adipose-mesenchymal stem cells can modulate plasma leptin and osteocalcin in polycystic ovary syndrome-induced rats

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Introduction: Animal models is useful for studying reproductive and metabolic abnormalities, including polycystic ovary syndrome (PCOS). Mesenchymal stem-cells (MSCs) can modulate biological pathways.
Objective: To investigate the modulation of the plasma leptin and osteocalcin by adipose-derived MSCs (AdMSCs) in an animal model induced to PCOS.
Methods: Adult female rats were divided into control (C, n=5), polycystic ovary (PCO, n=5), and MSC (n=5) groups, evaluated at two times (30 and 60 days) after PCOS induction and AdMSCs injection. The experimental procedures were approved by the Ethics Committee #030/2016. Data were analyzed using ANOVA and Fisher test. The results are reported as means ± SD, in ng/mL.
Results: 30 days: Plasma leptin was higher in the PCO group (13.33 ± 1.99), compared to the C (7.58 ± 1.1) and MSC (4.55 ± 0.145) groups. 60 days: There were reductions of plasma leptin in the PCO (3.49 ± 0.158) and MSC 2.44 ± 0.508) groups, compared to the C group (7.37 ± 1.078). In all the groups, osteocalcin was reduced at 60 days, compared to the 30 days (C = 0.099 ± 0.008 x 0.065 ± 0.0102; PCO = 0.088 ± 0.01 x 0.651 ± 0.01; MSC = 0.036 ± 0.002 x 0.014 ± 0.0018, respectively, 30 x 60 days).
Conclusion: The results suggest the existence of endocrine-metabolic-reproductive microenvironment relationships modulated by AdMSCs in an animal model induced to PCOS. This should aid in guiding further investigations to clarify pathophysiological mechanisms that have not yet been fully elucidated.
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Understanding the mechanism of a novel anti-obesity drug.

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Introduction: Obesity-related type II diabetes has dramatically increased global morbidity and mortality. Previously, the salicylate demonstrated promise of treatment for type II diabetes, but its clinical use was precluded due to high dose requirements and concomitant side effects.
Recently, we showed that the nitroalkene group of unsaturated nitro-fatty acids can be attached to different molecular scaffolds to confer beneficial drug actions. Taking advantage of this concept, we synthesized a novel nitroalkene derivative of salicylate that we called SANA.
Objectives: To evaluate the functional and metabolic effect of SANA in a diet induced obesity model.
Methods: C57BL/6J adult mice were fed with a high-fat-diet for 8 weeks and treated with SANA (or Salicylate) according to the approved bioethics protocol CEUA-IPMon 003–19. Weight, food-intake, glucose, insulin, triglycerides, free-fatty-acid, O2 and CO2 consumption were measured. Proteomics, metabolomics and mRNA expression analysis, as well as mitochondria respiration from white adipose tissue were performed. Statistical analyses: OW or TW ANOVA. Data is presented as mean ± SEM.
Results: SANA effectively prevented obesity and alleviated metabolic consequences of established obesity, hyperglycemia and hepatic steatosis. Proteomic, metabolomic and mRNA expression analyses of adipose tissue revealed SANA-mediated stimulation of catabolism, mitochondrial respiration and non-shivering thermogenesis. Indeed, SANA stimulated the recently described creatine-driven heat production pathway evidenced by elevation of tissue-specific creatine synthesis whereas depletion of creatine resulted in the absence of SANA-driven effects.
Conclusion: Taken together, our findings reveal that SANA promotes creatine-dependent thermogenesis which is a suitable pharmacological target for prevention and treatment of obesity.
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Gestational chronodisruption effects on offspring: Sex-related disparities in inflammatory and metabolic profiles in the rat

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Introduction: Chronodisruption, due to shift work and changes in photoperiod, exacerbates proinflammatory cytokine production, increasing the risk of NCDs. Remarkably, gestational chronodisruption has the potential to imprint circadian rhythms onto offspring, setting inflammaging, with deleterious effects on White Adipose Tissue (WAT).
Objective: We aimed to investigate the impact of maternal chronodisruption on the inflammatory and metabolic profiles of offspring.
Methods: Pregnant rats (sperm positive, at 12:12 photoperiod; LD) were separated into: A) Control (LD; n=8); B) chronic phase shift photoperiod (CPS; n=8); and C) CPS mothers supplemented with melatonin in the drinking water during night (CPS+Mel; n=8). At 18 days of gestation, pregnant dams returned to LD photoperiod. In the offspring

(90-400 days), we evaluated weight gain, WAT depot, glucose tolerance, and AM/PM plasma levels of cytokines, corticosterone and melatonin. The values were analyzed through one- or two-way ANOVA and expressed as mean±SEM, Protocols were approved by IACUC-UACh#CBA-352.

Results: CPS Male offspring display increased body weight than LD and CPS+Mel. With higher total fat content an elevated glucose response to fasting and intraperitoneal glucose challenges, peaking in the Area Under the Curve of the glucose challenge by 400 days. Females remained unaffected in this regard. Male and female CPS offspring showed diminished day/night differences in plasma melatonin and corticosterone, with unbalanced pro and anti-inflammatory cytokines (IL1a, IL6, and IL10). Maternal melatonin treatment reversed the effects on male metabolism and on female and male inflammation.

Conclusions: Our findings support that gestational chronodisruption programs low-grade inflammation in the offspring impinging a metabolic dysfunction in males

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Active role of the liver and adipose tissue in creatine-dependent thermogenesis: relevance of one-carbon metabolism

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Introduction: Obesity is a chronic metabolic disorder and a factor that predisposes to the development of metabolic syndrome, type II diabetes, and cardiovascular diseases. Addressing obesity has spurred interest in developing pharmacological tools to stimulate energy expenditure through thermogenesis, offering a potential approach to treatment. Thermogenesis is an active physiological response, prompted by beta-adrenergic stimulation. Recently, a thermogenic creatine-dependent pathway has been identified in brown and beige adipose tissues. Endogenous creatine is primarily synthesized in the liver and requires glycine, arginine, and methionine. Methionine, contributing its methyl group to creatine synthesis, needs to be recycled within the one-carbon metabolism. Both the transport of creatine and its localized synthesis within adipose tissues are likely to contribute to the thermogenic response. However, how the liver adapts to meet creatine demands remains unknown.

Objective: To assess liver and adipose tissue responses regarding creatine synthesis and methionine recycling during cold-induced thermogenesis.

Methods: We conducted cold-challenge experiments, subjecting C57Bl/6 mice to 4°C for 48 hours. We measured metabolite and mRNA levels of relevant enzymes. Statistical analyses employed t-tests and ANOVA, with results reported as mean±S.E.M. (n=5-25), conducted in accordance with Ethics Committee Protocol CEUA-IPMon 003-19.

Results: We observed an upregulation of genes linked to creatine synthesis and methionine recycling in both the liver and adipose tissue. Furthermore, there was a significant increase in both hepatic and plasma creatine levels.

Conclusion: Our study provides evidence that the liver and adipose tissue play pivotal roles in creatine synthesis and methionine recycling during the cold-induced thermogenesis.

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The methyl-CpG-binding protein-2 mediates hypothalamus-adipose tissue communication and determines metabolic phenotype

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Introduction: The hypothalamus is the central brain area controlling body energy balance, which requires maintaining high levels of plasticity to integrate and respond to peripheral signals that account for energy levels. This last requires a large amount of energy, primarily as ATP, by cellular energy metabolism. Mecp2 is a molecular bridge that binds to methylated CpG dinucleotides to orchestrate gene expression in response to environmental factors, and loss-of-function mutations cause overweight and mitochondrial dysfunction. However, the role of Mecp2 in controlling cellular energy metabolism and its impact on hypothalamic function still need to be understood entirely.

Objective: To evaluate cellular and physiological parameters associated with energy sensing and body energy metabolism.

Methods: The metabolic phenotype of Mecp2-KO mice was determined by evaluating body weight gain, energy intake, expenditure, respiratory exchange ratio (RER), and adipose tissue lipid composition. Mitochondrial function was measured by calcium buffering, energy production, and protein expression. Results are presented as Mean ± SEM and compared by ANOVA and t-test (CEC-USS 02022022).

Results: The lack of Mecp2 alters the hypothalamic expression of proteins involved in mitochondrial function and cellular energy metabolism. Besides, we found increased adiposity associated with changes in the expression of genes related to lipid metabolism and lipid composition. In addition, Mecp2-null mice show a body energy homeostasis disruption reflected by reduced locomotor activity and increased respiratory exchange ratio.

Conclusion: Our results show that the absence of Mecp2 alters cellular energy metabolism in the hypothalamus, impacting white adipose tissue metabolic control and body energy homeostasis.

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P-40

Iodine intake based on a survey from a cohort of women at their third trimester of pregnancy from the Bosque County Chile

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Introduction: Iodine is essential for thyroid hormone synthesis, which are crucial during pregnancy and lactation for maternal and infant

well-being. In Chile, where the intake of iodine-rich foods is scarce, a program to iodize table salt exists but lacks proper monitoring.

Objective: To evaluate iodine intake in pregnant women at the third trimester from El Bosque, Santiago de Chile.

Methods: Chilean pregnant women were recruited at Centros de Salud Familiar. Blood and urine samples were collected for thyroid parameters and urine iodine concentration (UIC) analysis. Iodine intake was determined using two methods: theoretical estimated (tEIC) and calculated (cIC) from a 24-hour dietary recall. Food iodine content was acquired from the United States Department of Agriculture's database, whereas Chilean bread and milk were analyzed via Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). Ethical approval was obtained from the Ethical Committee of Servicio de Salud Metropolitano Sur (379/2020). Results are expressed as mean ± S.E.M. (n=26).

Results: Global mean of all thyroid parameters and UIC fell within normal ranges for pregnant women. However, tEIC (269.6±16.40 µg) and cEIC (222.6±16.64 µg) exceeded the recommended range (160-220 µg). Iodine intake derived mainly from salt, bread, and milk. However, we did not find correlation between iodine intake and UIC.

Conclusion: This study is the first to integrate iodine content data for Chilean bread and milk into the Food Processor Software. However, since iodine intake did not correlate with UIC, we emphasize the need for a better parameter to assess iodine intake in pregnant women.

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Exposure to gestational hypothyroxinemia impacts fetal growth and metabolic function in the offspring

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Introduction: Maternal thyroid hormones play a critical role in fetal development, and gestational hypothyroxinemia (HTX), characterized by reduced thyroxine (T_4) levels in pregnant mothers, is associated with adverse outcomes in offspring, such as small-for-gestational-age (SGA) and large-for-gestational-age (LGA) in newborns. Birth weight is a well-established predictor of future metabolic and endocrine health; however, the influence of HTX on offspring's metabolism remains poorly understood.

Objective: To assess the effect of HTX on offspring's birth weight and long-term metabolic functioning.

Methods: Newborn pups (n=120, 60 pups/group) from control or HTX pregnant mice were classified as SGA or LGA based on z-score centiles. Subsequently, a subset of male mice, gestated or not in HTX, were subjected to either a cafeteria diet (CAF) or a standard diet (ND) for 10 weeks (n=32; 8 animals/group). We assessed weight gain, metabolic markers, and pancreatic insulin. Procedures were approved by the Bioethics Committee of the Life Sciences Faculty (005/2022). Data is presented as mean ± S.E.M. and statistical differences were assessed using Student t-test or Mann-Whitney for two-group comparisons and Two-Way ANOVA for multiple comparisons.

Results: A higher frequency of both SGA and LGA pups was observed in HTX-offspring. Moreover, HTX promoted weight gain, impaired glucose tolerance, and elevated triglyceride levels in adult offspring. Furthermore, exposure to a CAF diet exacerbated weight gain and elevated triglyceride levels in HTX-offspring.

Conclusion: This data highlights the potential contribution of gestational HTX to the development of overweight/obesity and metabolic disorders, emphasizing the role of early-life factors in shaping physiology.

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Sex-Specific Consequences of Placental Endocrine Malfunction on Mitochondrial Bioenergetics and Related Genes in Mouse Placenta

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Introduction: The placenta impacts maternal physiology both systemically and locally through hormone release, including the modulation of placental endocrine capacity and nutrient transfer, with mitochondria playing a role in steroidogenesis and energy production. **Objective:** Assess the impact of induce placental endocrine malfunction on mitochondria function in the endocrine junctional zone (Jz) and transport labyrinth zone (Lz) of the mouse placenta. **Methods:** Placental endocrine malfunction was induced by mis-expressing the imprinted Igf2 and H19 genes, responsible for placental endocrine cell formation and function (Jz-ICR1Δ). On pregnancy day 19, dissected Jz/Lz tissues were collected for mitochondrial respirometry and mitochondrial-related gene expression analysis. Analysis considered both fetal sexes, utilizing a two-way ANOVA with genotype and sex as variables. Data presented as mean ± S.E.M. Sample size: 6-9 per group for all experiments on each sex. Ethics committee code: UK HOL PP6324596. **Results:** Compared to controls, Jz from female Jz-ICR1Δ placentas displayed elevated Complex I Leak, Complex I+II Oxphos, and total electron transfer system respiratory rates. Male Jz-ICR1Δ placentas had unaltered respiratory capacity. Lz respiratory rates were unaffected in both sexes by Jz-ICR1Δ. Male Jz exhibited reduced expression of *Tfam*, *Nrf2*, *Opa1*, *Mfn1*, *Mfn2*, *Drp1*, and *Cyp1* due to Jz-ICR1Δ, while females showed no significant differences. Male Lz had increased *Pparδ*, *Mfn2*, *Drp1*, *Fis1*, and *Cyp1* expression, while only *Fis1* increased in female Lz due to Jz-ICR1Δ. **Conclusion:** Placental endocrine dysfunction induces sex-specific effects on mitochondrial bioenergetics and gene expression in the placenta.

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Inflammatory mRNA transcript expression in THP-1 macrophages exposed to extracellular vesicles from subjects with or without obesity

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Introduction: Obesity is a multifactorial disease characterized by a chronic low-grade inflammation, nonetheless molecular mechanisms that underlie metabolic alterations are not totally known. Peripheral blood mononuclear cells (PBMC) are circulating immune cells exposed to the systemic environment and a non-invasive proxy for assessing effects on metabolically-relevant organs. PBMCs release extracellular vesicles (EVs) which are an important means of paracrine and endocrine crosstalk. EVs are altered in obesity and may mediate obesity-related inflammation and cardio-metabolic disorders. **Objective:** To evaluate the proinflammatory mRNA response of THP-1 macrophages exposed to PBMC's EVs from individuals with (OB) or without (NOB) obesity. **Methods:** PBMCs were isolated from OB and NOB adults (StemCell Technologies protocol) (n=2). Conditioned media from PBMC culture were obtained to isolate EVs (Exo-spin™). THP-1 macrophages were exposed to an increasing dose of EVs (0.5×10^8, 1×10^8 and 2×10^8 particles/mL), LPS 10 ng/mL (positive control) or vehicle for 24 h (experiments in triplicate). Proinflammatory factor mRNA transcripts were evaluated by qPCR. Friedman test was applied. $p<0,05$ were considered significant. Subjects signed the informed consent (INTA's Ethics Committee #15-2021). **Results:** THP-1 cells exposed to EVs from OB showed a dose-dependent inflammatory response, while EVs from NOB showed no effect.

OB-derived EVs (2 x 10^8 particles/mL) exerted a 6.5, 6.8-, 4.5-, 3.9-, and 1,7-fold increase in TNF-α, IL-6, IL-1β, CCL2 and NLRP3 mRNA expression respectively over vehicle.

Conclusion: EVs from OB induced an inflammatory response in THP-1 macrophages while those from NOB had no effect.

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AREA: IMMUNOLOGY AND CANCER

P-44 ★ *undergrad sci competition*

Immunotherapy based on heat-conditioned melanoma cell lysate promotes the maintenance of stem-like CD8+ T cells by diminishing the acquisition of their exhausted state

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Introduction: $CD8^+$ T cell infiltration is crucial for the antitumor immune response. However, persistent tumor antigens stimulation and the tumor immunosuppressive microenvironment may lead to the development of an "exhausted" phenotype, decreasing its antitumor efficacy. However, expression of the transcription factor TCF-1 defines a subset of exhausted T cells with stem-like properties and is associated with an improved therapy response. We developed an immunotherapy called Tvax, based on heat-conditioned human melanoma cell lysate (TRIMEL) plus Concholepas-concholepas hemocyanin (CCH). Previous studies have shown that Tvax reduces tumor growth and increases the $CD8^+$ T cells infiltration. Interestingly, using B16.F10 lysate and CCH (B16vax) doesn´t have an antitumoral effect but does increase $CD8^+$ T cell infiltration, suggests that tumor infiltration is necessary but not sufficient for an effective response.

Objective: Tvax could preserve TCF-1 expression in $CD8^+$ T cells, allowing the maintenance of stem-like subsets and reducing the exhausted state.

Methods: Inoculated C57BL6 mice with 2.5x10^4 B16.F10 cells and then was immunized with Tvax, B16vax, or PBS. Statistical analysis used: One-way ANOVA with Tuckey's test or Kruskal-Wallis with Dunn's test. Bioetic protocol #1175 was accepted by University of Chile CICUA.

Results: Our results demonstrated that Tvax and B16vax treatments induced a significant decrease in the exhausted T cell. However, only Tvax showed a significant increase in TCF-1 and granzyme-B expression.

Conclusion: These findings suggest that immunization with Tvax induces specific phenotypic and functional characteristics in CD8+ T cells. This could, at least in part, explain the effective antitumor response observed with Tvax treatment.

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P-45

Functional relevance of HCN channels in astrocytoma and lung cancer cell models

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Introduction: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, a family of non-selective cation channels, are widely expressed in the central and peripheral nervous systems and cardiac tissues. They play a pivotal role in regulating various physiological functions. HCN channel family is comprised of four members, HCN1-HCN4, sharing ~60% sequence identity. Structurally, HCN channels belong to the superfamily of voltage-gated potassium channels (Kv) and can form homo- or heterotetrameric complexes, leading to distinct biophysical properties in different channel subtypes. Recent findings have shown an upregulation of HCN

channels in various types of human tumors, including those originating from brain and lung tissues. However, the functional significance of HCN channels in cancer hallmarks, such as sustained proliferation, migration, invasion and metastasis, remains unexplored.

Objective: To investigate the potential role of HCN channels in cancer, we assessed the expression of HCN channels in U373 and A549 cancer cell lines.

Methods: We employed immunocytochemistry, immunoblotting, and real-time PCR (qPCR) analysis to determine the gene and protein overexpression of HCN channels. Group differences were calculated with t-student. Differences with $p \leq 0.05$ were considered statistically significant, and all data were shown as mean ± standard error of the mean (SEM).

Results: Our results confirmed the presence of HCN2 and HCN3 in the brain and lung cell lines.

Conclusion: These findings suggest that HCN2 and HCN3 ion channels hold promise as potential candidates for cancer diagnosis and prognosis. Furthermore, specific knockdown of HCN2 or HCN3 channels may present a viable strategy for reducing the likelihood of tumor formation.

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P-46

Development of a microfluidic platform as an interaction interface for detection of extracellular vesicles derived from breast cancer cell lines

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Introduction: Cancer is the second leading cause of death at worldwide. Improving cancer survival rates globally requires improvements in early diagnosis and prediction of disease relapse. It is imperative much less invasive methods than conventional biopsy. Liquid biopsy refers to the sampling and analysis of non-solid biological tissue, most commonly peripheral blood, in which tumor-derived material may be found. Prior to metastasis, cancer cells release large amounts of extracellular vesicles (EVs) that contain tumor-specific markers which facilitate tumor development and metastasis. EVs in liquid biopsy represent a good choice for screening the presence of tumor markers on a non-invasive method.

Objective: Our goal is to design and produce a high-throughput microfluidic device, engineered with a 3D-nanostructure that ensures effective and specific contacts of surface protein of EVs from breast cancer cells from a liquid biopsy.

Methods: We fabricated a microfluidic device to capture EVs, using a microchip composed inside by a 3D nanostructure of monodisperse colloidal silica bioconjugated with specific surface protein antibodies of EVs (Lactadherin, CD81 and CD63) from breast cancer cell lines.

Results: We detected and quantified specific EVs derived from breast cancer cell lines in a 3D nanostructure platform bioconjugated with antibodies from surface proteins of EVs ($n=3$, $p<0.05$, t-test), validated by epifluorescense and scanning electron microscopy.

Conclusion: We are working on validating the efficiency of our microchip to detect EVs derived from breast cancer cell lines, as a new non-invasive, portable and user-friendly medical device, that offers more sensibility, rapid detection and potentially less expensive.

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P-47

Pharmacological study of Adenosine receptor A2BAR on autophagy-mediated chemoresistance in GSCs

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Introduction: Glioblastoma (GB) is an aggressive malignant tumor that has a survival rate of approximately 15 months. The standard treatment in chemotherapy is temozolomide (TMZ). However, the resistance and recurrence of

GB is attributed to glioblastoma-like-stem-cells (GSC) and they inhabit a tumor environment rich in adenosine, which activates low-affinity A2B receptors, and their activation is associated with proliferation and resistance to treatment with TMZ. The evidence found in our laboratory demonstrates that adenosine modulates autophagy. Therefore, we propose that pharmacological blockade of A2BAR causes chemosensitization of the U87 GSC cell line to TMZ by decreasing autophagic flux.

Objective: Evaluate whether pharmacological blockade of A2BAR causes chemosensitization to TMZ by decreasing autophagic flux in GSCs.

Methods: We determined the viability of GSC neurospheres subjected to treatments with 50 nM MRS1574 (A2BAR antagonist), 400 uM TMZ, and combinations, using MTS. Autophagic flux was assessed by Western blotting by determining LC3 II/I and p62 protein levels, which were analyzed by densitometry using ImageJ software.

Results: The data obtained show that at 48 hrs. (± 5.4 S.D.; n=3) and 72 hrs. (± 1.7 S.D.; n=3) chemosensitization is observed for combined therapy. The preliminary results of Western blotting showed accumulation of p62 at 48 hrs (1.953 A.U.) and 72 hrs. (1.488 A.U.), in addition to the variations in the LC3 II/I ratio at 48 hrs. (1.520 A.U.) and 72 hrs. (2.394 AU).

Conclusion: Pharmacological blockade could produce changes in autophagic flux that favor chemosensitization of GSCs.

Financing: Fondecyt regular 1200885 and Instituto Milenio de Inmunologia e Inmunoterapia (IMII).

P-48 ★ *undergrad sci competition*

The cGAS/STING signaling pathway: the key role in microglia function during aging

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Introduction: Aging is a natural process that often leads to a decline in brain function due to inflammation and neurodegeneration. Microglia, are important for brain maintenance and homeostasis, protect neuron survival, and induce inflammation in the presence of potentially dangerous stimuli. These microglia express almost exclusively the cGAS/STING signaling pathway, which is involved in the inflammatory response to cell damage, specifically when double-strain DNA from the nucleus and mitochondria is present in the cytosol.

Objective: We aimed to understand the role of the cGAS/STING pathway in microglia function during aging and how their absence might affect it,

Methods: we evaluated the levels of these proteins and different parameters in microglia and brain sections from different groups of mice—those with both proteins Wild Type (WT), those without cGAS (cGASKO), and those without STING (STINGKO) at diverse ages (accordance to the bioethics committee code 5/2018; Fondecyt 11190258). Statistical analyses were conducted using Two Way Anova with n=5-9 mice per experimental condition using mean ±SEM.

Results: Our tests showed that as brains age, the levels of cGAS and STING proteins decrease in the WT mice brain with age. We also observed early signs of aging in cGASKO and STINGKO mice, such as memory reduction behavioral, brain cell damage, and alterations in microglia, even at young ages compared to WT mice.

Conclusion: These results suggest that the cGAS/STING pathway is essential to maintaining brain homeostasis during aging, affecting microglia functions and, consequently, neuron survivance.

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AREA: NEUROPHYSIOLOGY

P-49

Anxious and depressive temperaments are associated with specific types of physiological response during verbal and visual memory tests

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Introduction: Declining in in cognitive functions related to memory is a common complaint in patients with anxious and depressive symptoms. The physiology of memory and learning is altered by phenomena in the psychiatric sphere. 20

healthy subjects aged 17 to 25 years were recruited to evaluate those variables.

Objective: To assess how individuals with higher scores on the Goldberg scales for anxious and depressive symptoms, as well as those exhibiting predominantly anxious or depressive temperaments, fare in verbal and motor learning tasks.

Methods: Participants underwent comprehensive assessments for verbal and motor learning. Additionally, anxiety and depression traits were measured using the Goldberg questionnaire. Visual analysis of heart rate and respiratory frequency records was conducted. Statistical analysis was performed with spss. Pearson correlation was used to evaluate the correlation between continuous variables. The subjects gave informed consent. The protocol was submitted to the ethics committee of the Faculty of Medical Sciences, Bernardo O'Higgins University.

Results: Subjects with elevated scores on the Goldberg scales indicative of anxious and depressive symptoms, along with those displaying predominantly anxious or depressive temperaments, demonstrated lower performance in both verbal and motor learning tasks dependent of working memory functions dependent of depresion and anxiety level (F= 4.34, Rsquared=9.19). Notably, visual analysis of physiological records revealed distinct patterns in individuals with diminished task performance.

Conclusions: anxiety and depression, as well as temperamental tendencies modify verbal and motor learning abilities in young adults. The integration of physiological data provides additional insight into the mechanisms associated with task performance disparities.

P-50 ★ *undergrad sci competition*

Functional evaluation of hHCN3 channel mutations associated to Attention-Deficit Hyperactivity Disorder (ADHD) and psychotic disorder

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Introduction: Hyperpolarization-activated and cyclic nucleotide (HCN)-modulated channels are nonselective cation channels encoded by four genes (HCN1 to HCN4). They regulate different physiological functions, being widely expressed in the central and peripheral nervous systems and in cardiac tissues. Dysfunctional HCN channels have been implicated in pathological processes such as schizophrenia and mood disorders. To date, no HCN3 mutations have been described in pathologies associated with neuropsychiatric disorders.

Objective: The aim of the research is focused on characterizing two heterozygous mutations (V475M and H573Y), recently detected in a patient, presenting ADHD, social communication disorder and psychotic disorder factors.

Methods: COS-7 cells were co-transfected with the cDNAs coding for the wild-type and mutated channels, together with specific membrane and endoplasmic reticulum cell markers. In an in silico approach, a homology model was generated from the HCN4 channel (85.21% identity) and subsequently, the mutations were introduced.

Results: Co-localization results were analyzed by Pearson correlation, revealing an increased distribution of the mutated channels in the endoplasmic reticulum, suggesting a connection between mutations and channel retention. In the homology model, the V475M mutation is located in the CNBD and H573Y in the C-terminal region.

Conclusions: Preliminary results suggest that the mutated channels could retain their transporter function, taking into account that the mutations do not affect the regions close to the pore. However, their cellular distribution would not be mainly in the membrane, which could imply a reduction of activity at the cellular level. This will be analyzed by patch clamp in whole cell configuration.

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P-51

Effects of Thyroid Hormone T3 in an Alzheimer's disease-like experimental model

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Introduction: Alzheimer's disease (AD) is a common, incurable neurodegenerative condition. Thyroid hormones (T3 and T4) play a vital role in maintaining metabolism and cognitive health in aging, facilitating processes like neurogenesis, myelination, and cell repair.

Objectives: To evaluate the effects of triiodothyronine (T3) thyroid hormone supplementation in cognitive performance related to animals' memory and neuroinflammation.

Methods: Male Wistar rats, received bilateral injections of vehicle or streptozotocin (STZ) in the lateral ventricle by stereotaxic surgery to induce AD model. The animals were supplemented with a daily dose of 1.5 µg/100g of T3 or the same volume of vehicle, for 30 days. They Were evaluated for motor activity, and cognitive memory performance in novel Object Recognition Behavioral Test. Neuroinflammation was assessed by Western Blot and Microglial marker (IBA1), astroglia marker (GFAP), and interleukin 1 beta (IL-1β) in the hippocampal CA1 region were examined by immunohistochemistry. All the experiments were approved by animal ethical committee (CEUA) (nº 023/2016). Data analysis involved a two-way analysis of variance (ANOVA) with Tukey's post-test for specific group comparisons. The data were reported as mean ± SEM, and a significance level of 5% ($p < 0.05$) was applied.

Results: T3 supplementation promotes improvement in cognition in the object recognition index of short and long-term tests and reduced the GFAP, IBA1, and IL-1β positive cells. T3 also reduced GFAP and TNF-α immunodetected levels in animals with AD.

Conclusion: Our data provide evidence of the positive effects of T3 supplementation in cognition and neuroinflammation in a model of AD.

Financing: ANID, Becas Chile

P-52

Chronic exposure to a high-fat diet affects the activity and neurochemical regulation of the lateral septum

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Introduction: In obesity, hyperphagia disrupts cerebral circuits regulating food intake. Additionally, the inhibitory nucleus lateral septum (LS) plays a role in physiological processes such as cognitive function and autonomic control. Excitatory signals from the hippocampus (HP) to the LS and GABAergic projections from the LS to the lateral hypothalamus (LH) and ventral tegmental area (VTA) are pivotal in food control.

Objective: To evaluate the impact of a high-fat diet (HFD) on the neurochemical and electrophysiological properties of the LS.

Methods: 72 Sprague-Dawley rats were exposed to either a standard or HFD from PND 21 to 62. Gene expression of Dopamine receptors, GABA, and the DAT transporter in the LS was quantified using qRT-PCR. Dual microdialysis measured GABA in the LS and LH using HPLC. Horizontal brain slices recorded fEPSPs in the LS, evoked by paired pulses in the fiber pathway from CA3 of the HP. CA1 fEPSPs were recorded in coronal sections of the dorsal hippocampus. Data presented as means ± standard error; statistical Shapiro-Wilk, two-tailed t-test, Mann-Whitney, and ANOVA with Bonferroni post hoc correction. Significant differences at $p < 0.05$. BioethicsBEA154-20.

Results: Extracellular GABA levels induced by potassium perfusion were reduced in the LS and LH of rats exposed to HFD. Paired-pulse ratio (PPR) analysis in the LS showed a higher PPR in the HFD group. In CA1, decreased fEPSP slopes were observed in HFD animals, along with a lower population spike threshold in this group. There were no differences in Dopamine receptors, GABA, and the DAT transporter gene expression.

Conclusion: HFD alters the activity and neurochemistry of septal neurons.

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P-53

Role of astroglial gliotransmission in memory, stress and depression

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Introduction: Astrocytes modulate glutamatergic synaptic transmission by releasing neuroactive molecules (gliotransmitters) onto synapses, like glutamate, D-serine and ATP, mainly via connexin 43 hemichannels (Cx43 HCs). Howerver, the role of astroglial gliotransmission in higher brain function and pathology is only beginning to emerge.

Objective: To determine the role of astroglial Cx43 hemichannel-dependent gliotransmission in memory, stress and depression.

Methods: We used a combination of in vivo intracranial microinjections, ex vivo (slice) preparations and in vitro techniques, using Cx43 hemichannel blockers and gliotransmitters, as well as measuring extracellular glutamate and ATP, to study the role of Cx43 hemichannels in fear memory, acute stress and chronic restraint stress-induced depression. This study was approved by UNAB´s bioethical comittee. All experiments had an n>3.

Results: We found that in the basolateral amygdala, the astroglial Cx43 HC-dependent release of D-serine and glutamate regulates the activity of post-synaptic NMDARs required for the formation of short-term fear memory and subsequent memory consolidation, but not for learning. In the ventral hippocampus, we found that astroglial Cx43 HC activity increases after chronic stress, leading to the enhanced release of glutamate, ATP and D-serine, which trigger the development of depressive symptoms via increasing postsynaptic NMDAR activity.

Conclusion: Astroglial gliotransmission appears to be pivotal in regulating the NMDAR-dependent glutamatergic transmission that is required for memory and stress responses, but is also critical for triggering depressive symptoms after chronic stress.

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P-54 ★*selected for oral communication*

Changes on microglia-neuron interaction in aging and neuroinflammation

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Introduction: Changes in glial cell activation are observed in aging and neuroinflammation. They depend on multiple factors, including changes in transforming growth factor (TGFβ) and Scavenger Receptor A (SRA) signaling. Those changes can also affect neuron-microglia regulatory crosstalk, in turn participating in the regulation of glial cells cytotoxicity.

Objective: Fractalkine/CX3CR1 is involved in the regulation of microglia by neurons. Our results show that age-related changes result in the modification of glial cells regulation. Here, we assess age-dependent changes of various pathways in the brain of wild type (WT) and a mouse knockout for SRA (SRA-KO).

Methods: Bioethics approval CA1495144788105 Pontificia Universidad Católica de Chile and CECUA (28042022) from Universidad San Sebastian. We analyzed 3-6-, 12- and 20-month-old mice. To assess the effect of inflammation, mice received 1 mg/kg intraperitoneal LPS or vehicle. After 2-to-24h, fractalkine and CX3CR1 were assessed by qRT-PCR and western blot. two-tailed Mann-Whitney test was used. Data are mean±SEM. The distribution of receptors and signaling pathways activation were assessed by immune histochemistry.
Results: We observed that TGFβ, activation of inflammatory signaling pathways (1-to-3-fold change; $p<0.01$; n=7), SRA (60% decrease; $p<0.001$; n=7) and fractalkine were affected by aging, even in the absence of exogenous inflammation. Several changes are already observed at 12 months, including a 3-fold increase of 70 kDa Fractalkine ($p<0.01$; n=5) and the appearance of 40 kDa Fractalkine (not observed in younger mice) compared with young mice, with predominance of soluble isoforms.
Conclusion: Aging favors the generation of soluble (cleaved) Fractalkine, promoting microglial activation and neuroinflammation.
Financing: Grants FONDECYT 1211359, 1221028 and ANID-REDES 190178

P-55

Neuronal glutamate transporter EAAT3 regulates heterosynaptic GABAergic plasticity and reversal learning

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Introduction Long-term depression (LTD) is a form of synaptic plasticity that plays an important role in tasks involving modification or elimination of previously learned information. Here we show that neuronal glutamate transporter (EAAT3) controls a form of heterosynaptic and endocannabinoid-dependent LTD at inhibitory synapses ("i-LTD") in the CA1 region of the hippocampus and contributes to the modulation of reversal learning.
Methods Electrophysiological recordings in mouse brain acute slices from 4-5 animals for each experiment and the Morris water maze for cognitive evaluation (n=5) were performed. Analysis was done using t-test. All procedures were approved by the University of Valparaiso Bioethics committee (BEA159-20).
Results Overexpression of EAAT3 in principal cells does not alter the strength or short-term plasticity of excitatory synapses but significantly impairs i-LTD. Importantly, i-LTD alteration was independent of both endocannabinoid and mGluR signaling, and absent when EAAT3 was overexpressed at GABAergic interneurons; also, it can be rescued by pharmacologically blocking EAAT3 but not EAAT2 transporter, strongly suggesting that EAAT3 controls the escape of glutamate from excitatory synapses to neighboring GABAergic synapses to regulate hippocampal i-LTD. Behaviorally, we found no detectable changes in the acquisition phase of spatial learning when EAAT3 was overexpressed. However, a marked impairment in reversal learning was observed only in mice overexpressing EAAT3 at principal cells.
Conclusions Altogether our data support the notion that EAAT3 regulates the strength and the extent of receptor activation by afferent activity, and thus controls a form of heterosynaptic LTD at inhibitory synapses and contributes to regulate cognitive flexibility processes.
Financing: This work was supported by the Chilean government through FONDECYT Regular #1201848(AEC), #1231012(PRM). CAG was supported by a PhD fellowship #21201603 from ANID.

P-56

Role of Pannexin 1 in the initiation of central spinal sensitization and its interaction with the TrkB receptor in the rat spinal cord

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Introduction: There is evidence that shows the participation of brain-derived neurotrophic factor (BDNF) (Constandil, L. et al, Journal of Pain,2012, https://doi.org/10.1016/j.jpain.2012.03.008) and Pannexin1 (Bravo, D. et al, Pain, 2014, https://doi.org/10.1016/j.pain.2014.07.024

) in the initial stages of the central sensitization process.

Objective: To determine if there is an interaction between the TrkB receptor and the Pannexin1 channel during the onset of BDNF-induced central sensitization.

Methods: Evaluation of the algesimetric behavior of adult male Sprague-Dawley rats, using the Randall-Selitto test. Data expressed as ± SEM. N:6 for each group. Project approved by the Bioethics and Biosafety committee of the University of Santiago Nº 626.2022.

Results: A decrease in the paw withdrawal threshold of 22.49 ± 6.308 gr/cm2 was observed for the experimental group, which began 5 minutes after BDNF administration. A decrease of 46.59 ± 11.48 gr/cm2 was generated during the 10 days of evaluation. Regarding the group that received 10Panx prior to the administration of BDNF, we can mention that during the first 4 hours an increase of 40.16 ± 9.423 gr/cm2 is generated, which subsequently decreases, returning to values close to baseline on day 10 of the evaluation. These changes were accompanied by molecular and functional modifications, such as increased phosphorylation of Pannexin1 and Src.

Conclusions: We can conclude that the intrathecal administration of BDNF generates an initial sensitization of rapid onset that is maintained for at least 10 days. This phenomenon is partially reversed by using the Pannexin1 channel blocker called 10Panx.

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Effects of Schwann cell-derived exosomes on neuropathic pain in a model of chronic constriction injury

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Introduction: Entrapment neuropathies are painful diseases caused by mechanical nerve compression. Pathophysiology includes demyelination, small fiber neuropathy and neuroinflammation. After nerve injury, Schwann cells (SC) are reprogramed into a repair state and release exosomes charged with molecules that promoted axonal regeneration and immunomodulation.

Objective: We hypothesize that local treatment with SC exosomes after a chronic compression injury (CCI) has beneficial effects on neuropathic pain and nerve repair.

Methods: We first characterized SC- derived exosomes cargo by qPCR. Then, we studied the effects of SC-derived exosomes in a rat model of sciatic nerve CCI. A single dose of SC-derived exosomes was injected in the sciatic nerve 4 days after CCI. Mechanical and thermal hypersensitivity were assessed using the electronic von Frey instrument and radiant heat application until 14 days post-injury. Statistical analysis was performed using the mean ± S.E.M of 8 rats per condition. A P-value of <0.05 was considered statistically significant from Two-way ANOVA followed by Tukey's post hoc test. Ethical protocol was approval by Pontificia Universidad Católica Ethics Committee, 220617028.

Results: We showed that SC-derived exosomes cargo contains neurotrophic factors and inflammatory mediators. A single dose of SC-derived exosomes significantly decreased the mechanical and thermal hypersensitivity developed after injury. The alleviation in the hypersensitivity was more effective for mechanical (vehicle 15g±3 vs exosomes 29g ±2; withdrawal threshold (g)), then thermal stimulation.

Conclusions: SC-derived exosomes improved the mechanical and thermal hyperalgesia after a nerve chronic constriction and suggests a promising potential for the use of these nanovesicles as a safe therapeutic strategy to improve neuropathic pain.

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Effects of intrahippocampal vasopressin administration on amphetamine-induced addictive-like behavior and neurochemical effects on the reward circuit

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Introduction: There are sexually dimorphic extrahypothalamic vasopressinergic projections that regulate different responses, including addictive behavior. On the other hand, the involvement of the hippocampus during the development and maintenance of addictive behaviors has been demonstrated through projection to the lateral septum (LS) and that it also receives considerable vasopressinergic projections from extrahypothalamic areas. However, the role of AVP in the dorsal hippocampus in addictive behaviors has barely been studied.

Objective: The aim of this research was to study sex differences in the effects of AVP in CA3d on amphetamine (AMPH)-induced place preference conditioning and neurochemical responses of the reward system in male and female Sprague Dawley rats (bioethics N°: 204/2022).

Methods and Results: We observed that intra-CA3d AVP microinjection reduces AMPH-induced CPP in both sexes (females: (AVP: t(18)= 0.2517, p=0.4021; VEH: t(20)= 3.355, p=0.0016), males: (AVP: t(20)= 0.7677, p=0.2258; VEH: t(24)= 2.904, p=0.0039)). However, intra-CA3d AVP administration decreases NAc dopamine (DA) tissue levels in female rats, with no changes in NAc DA tissue levels in male rats (females (t(8)= 2.723, p= 0.0261), males(t(16)= 0.9511, 0.3557)). On the other hand, no changes were observed in the extracellular levels of GABA and glutamate in the lateral septum (LS) of male and female rats after intra-CA3d AVP microinjection.

Conclusion: The administration of AVP in CA3d produces the same effect in male and female rats on the expression of addictive-like behaviors and neurochemistry in the LS. These results contribute to the knowledge of the potential therapeutic effect of AVP on addiction to AMPH.

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P-59

Contribution of Neuronal Surface P Antigen (NSPA), a protein that is required for N-methyl-D-aspartate receptor (NMDAR) function, in energy homeostasis. Metabolic characterization of NSPAKO mice

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Introduction: At the central level, energy homeostasis is mainly controlled by the hypothalamus through AGRP and POMC neurons. Hypothalamic NMDARs influence energy balance; GluN2B subunit of NMDAR in AGRP neurons is required for normal control of calorie intake and body weight, playing a critical role in energy balance. Evidence from hippocampus shows that tyrosine phosphorylation of the intracellular domain of GluN2B, particularly Tyr1472 controls NMDAR trafficking, location, and protein-protein interactions, preventing NMDAR endocytosis. NSPA protein is required for NMDAR function and synaptic plasticity, its absence decreases NMDAR-mediated transmission and synaptic plasticity in the hippocampus translating into impaired spatial memory. The lack of NSPA decreases the phosphorylation of GluN2B-Tyr1472 and NMDAR levels at the hippocampal PSD.

Objective: To evaluate the contribution of NSPA to hypothalamic function and energy homeostasis.

Methods: Comparative studies in male WT and $NSPA^{KO}$ mice fed (7 weeks) with chow or HFD diet, on glucose and insulin tolerance, metabolic respiratory exchange ratio as an indicator of energy expenditure, feeding, and locomotor activity. Ethical Committee approval 07-2021-10.

Results: $NSPA^{KO}$ mice fed with chow diet showed higher respiratory exchange ratio (RER) and horizontal locomotor activity (n=5, Mann-Whitney Test), without significant changes in body weight, food intake and glucose and insulin tolerance at 10 weeks of age.

Conclusion: We observed that $NSPA^{KO}$ mice showed increased RER at 10 weeks, an elevated RER indicates decreased fat oxidation, which can lead to an increase in fat accumulation. We have preliminarily observed in $NSPA^{KO}$ mice higher fat mass, suggesting a role of NSPA in energy balance.

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Chronic intermittent hypoxia alters GABAergic inhibition in median preoptic nucleus (MnPO) neurons of rats

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Introduction: Chronic intermittent hypoxia (CIH) is an animal model simulating the hypoxemia associated with sleep apnea. Male rats exposed to CIH exhibit elevations in blood pressure. In MnPO neurons, CIH reduces GABAa inhibition and, in some neurons, converts it to excitation.

Objective: Here, loose patch and perforated patch recordings were conducted in MnPO neurons in response to optogenetic stimulation of GABAergic SFO and MnPO neurons.

Methods: Using isoflurane (2-3%) anesthesia, male Sprague-Dawley rats (250-350g) received infusions (0.2 µL) of pAAV-mDlx-ChR2-mCherry-Fishell-3 in the SFO or MnPO. After recovery, rats were subjected to 7 days of CIH (0800-1600 hr) or normoxia (Norm). CIH consisted of 6 min cycles (3 min 21% O2, 3 min 10% O2) repeated 10x/hr for 8 hours (during the normal inactive/sleep phase) on 7 consecutive days. Rats were anesthetized with isoflurane (2-3%) and sagittal or coronal slices (300 µm) containing MnPO were cut using standard slice procedures. Voltage-clamp loose patch or perforated patch recordings using gramicidin (100 ug/ml in 140 mM K-gluconate) were collected in MnPO neurons and analyzed using One-way ANOVA with values reported as mean ± S.E.M.

Results: MnPO neurons from CIH-treated rats showed differing responses to GABAergic stimulation while MnPO neurons from Norm-treated rats predominantly exhibited GABAergic inhibition. MnPO neurons from Norm rats exhibited mIPSCs and outward currents in response to muscimol. MnPO neurons from CIH-treated rats show variable mIPSC frequencies and heterogeneous responses to GABAergic neuronal activation in the SFO and MnPO.

Conclusions: CIH alters GABAergic stimulation in MnPO neurons and that may contribute to hypertension.

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Pannexin 1 channel blockade with Probenecid modulates glutamatergic signaling in hippocampal culture neurons of Alzheimer's disease murine model

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Introduction: Alzheimer's disease (AD) is a neurodegenerative condition that affects memory and learning in elderly individuals due to decreased glutamatergic neurotransmission and synaptic plasticity (SP). Pannexin 1 (Panx1) is a transmembrane protein that plays a role in hippocampal long-term depression (LTD) and is increased in an AD murine model (APP/PS1).

Objective: In this study, we investigated the contribution of Panx1 to the glutamatergic synapses under resting and SP conditions in hippocampal neurons.

Methods: We use postnatal hippocampal cultures from WT and APP/PS1 mice incubated at day in vitro (DIV) 14 with vehicle or Probenecid (PBN), a known Panx1 blocker, to measure the expression and activity of glutamatergic machinery by western blotting, ethidium uptake, pharmacology, immunocytochemistry. All animal manipulations were approved by the Ethics and Animal Care Committee of Valparaíso University (BEA160-20).

Results: We found an increase in the expression and activity of Panx1 channels in APP/PS1 neurons compared to WT group (2.547±0,2532, n=36; vs. 1.00±0,05833, n=21). Furthermore, we observed, under resting conditions, an increase of total GluR1 (0.5815±0,2419, n=13; vs. 1.476±0,1881, n=4) and VGluT1 (0.5166±0,04482, n=3; vs. 1.163± 0,1864; n=4) proteins in neurites of APP/PS1 neurons treated with PBN, while, after

chemical induction of long-term potentiation (LTP), our preliminary results showed an increase in the surface level of GluR1 compared to WT conditions. The results were expressed as mean±S.E.M and statistical analysis was performed using one-way ANOVA.

Conclusions: These findings suggest a novel role for Panx1 channels in modulating glutamatergic signaling of hippocampal neurons in the AD model.

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The guanine nucleotide exchange factor RapGEF2 is required for ERK-dependent immediate-early gene (Egr1) activation during fear memory formation

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Introduction: RapGEF2 is a neuron-specific cAMP sensor that mediates ERK activation, and ERK is important for neuronal plasticity underlying associative learning.

Objective: We identified whether RapGEF2 is required for ERK activation leading to other downstream neuronal signaling events during fear memory formation.

Methods: RapGEF2 expression is ablated in excitatory neurons of hippocampus and basolateral amygdala (BLA) in CamK2α -Cre::RapGEF2$^{fl/fl}$ mice. We used these mice to assess the role of RapGEF2 in long-term post-synaptic potentiation, in both the hippocampal Schaffer collateral and perforant pathways, and in contextual or cued fear memory formation. ERK activation and immediate early gene induction in hippocampus and BLA during fear learning were also examined. Animal studies was approved by the NIMH Animal Care and Use Committee.

Results: CamK2α-Cre::RapGEF2$^{fl/fl}$ mice showed impaired cAMP-dependent long-term potentiation at perforant pathway and Schaffer collateral synapses in hippocampal slices ex vivo; impaired contextual memory consolidation; and abolition of pERK and Egr1, but not Fos induction, following fear conditioning. CamK2α-Cre::RapGEF2$^{fl/fl}$ mice also showed impaired cue-fear memory, associated with reduced Egr1 (but not Fos) induction in BLA after restraint stress-augmented fear conditioning. Impairments were assessed by appropriate statistical methods, at $p<0.05$ or less.

Conclusion: A RapGEF2→ERK→Egr-1 signaling pathway in hippocampus and BLA is required for fear learning. The parcellated signaling model points towards a mechanism through the simple expedient of segregation of regulation of one set of immediate early genes (typified by Egr1/Zif268) by one cognitive kinase, ERK, from another set of immediate early genes (represented by fos induction) by other cognitive kinases.

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AREA: RENAL AND GASTROINTESTINAL

P-63 ★*selected for oral communication*

Enhancing renal graft function and alleviating ischemic kidney injury through synchronized modulation electric field for Na+/K+-ATPase maintenance

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Introduction: Renal ischemia-reperfusion injury is an important contributor to the development of delayed graft function. One of the earliest impairments during ischemia is Na+/K+-ATPase dysfunction due to insufficient ATPs, resulting in subsequent cellular damage. Therefore, strategies

that maintain Na/K pump function may limit the extent of renal ischemic injury.

Objective: Here, we presented a technique using a synchronization modulation electric field (SMEF) to activate Na/K pumps, thereby maintaining cellular functions under ATP insufficient conditions.

Methods: We tested the effectiveness of this technique in a renal ischemia-reperfusion injury model and a kidney transplantation model. All procedures and experiments were performed with the approval of the Institutional Animal Care and Use Committee at the University of South Florida (IACUC, IS000011615R). All values are presented as means ± SEM. Comparison of the data was performed using one/two-way ANOVA followed by Tukey's multiple comparison test.

Results: Our results showed that application of SMEF preserved Na/K pump activity, thereby alleviating kidney injury reflected by 40% lower plasma creatine (1.17±0.03 mg/dL, n=5) in the treated group than control group (1.89±0.06 mg/dL, n=5) in a mouse renal ischemia-reperfusion model. In the mouse kidney transplantation model, renal graft function was improved by 54.20% with the application of SMEF according to the GFR measurements (85.40±5.44 µl/min for the untreated group and 142.80±5.21 µl/min for the treated group).

Conclusion: This technique for preserving Na/K pump function may have therapeutic potential not only for ischemic kidney injury, but also for other diseases associated with Na/K pump dysfunction due to inadequate ATPs.

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Caveolin-1 and Caveolin-2 as potential urinary early biomarkers of renal injury in acute cholestasis in rats

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Introduction: Renal injury is a main complication in cholestasis. Conventional biomarkers for kidney function such as plasma urea levels (U_p) and glomerular filtration rate (GFR) have a diagnostic value when renal function has already lost. Our group has worked in the search for urinary biomarkers that could early indicate the presence of renal dysfunction in other pathologies.

Objective: The aim of this work was to evaluate the urinary excretion of renal tubular proteins, Caveolin-1 (Cav-1_u) and Caveolin-2 (Cav-2_u) as potential biomarkers of early renal damage induced by obstructive cholestasis in male Wistar rats.

Methods: Obstructive cholestasis was surgically induced by Bile Duct Ligation (BDL) for 21h (BDL21h, n=5) and 72h (BDL72h, n=6). Sham-operated control group (Sham, n=7) was also processed (Institutional Animal Care and Use Committee, FBIOyF-UNR, Res. 657/2016). Up was determined; GFR was calculated by creatinine clearance (CrCl). The urinary excretion of Neutrophil Gelatinase-Associated Lipocalin ($NGAL_u$), a novel urinary biomarker of kidney injury, Cav-1_u and Cav-2_u were evaluated by immunoblotting. ANOVA-Newman Keuls, P<0,05: (a) vs. Sham, (b) vs. BDL21h, (c) vs. BDL72h.

Results: (Mean±SEM) U_p(g/L) Sham=0.44±0.03, BDL21h=0.41±0.02, BDL72h=0.45±0.04; CrCl (mL/min.100g): Sham=0.44±0.02, BDL21h=0.47±0.02, BDL72h=0.35±0.01^{a,b}; Cav-1_u(%): Sham=100±2, BDL21h=145±14^a, BDL72h=145±8^a; Cav-2_u(%): Sham=100±3 , BDL21h=138±8^a, BDL72h=133±8^a; $NGAL_u$(%): Sham=100±3, BDL21h=118±8, BDL72h=124±9^a. U_p was not altered while CrCl and $NGAL_u$ were significant increased after 72h of BDL. Cav-1_u and Cav-2_u were significant increased at 21h after BDL.

Conclusion: These results provide evidence for Cav-1_u and Cav-2_u as potential early biomarkers of renal damage induced by obstructive jaundice. This could be a strategy to predict cholestasis-induced renal injury.

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Distant organ dysfunction in acute kidney injury induced by kidney ischemia-reperfusion. Alteration of ABC transporters expression in the liver

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Introduction: Kidney ischemia-reperfusion (IR) is a complex pathophysiological process that affects many organs besides kidneys. ATP-binding cassette (ABC) transporter superfamily is involved in the efflux of endogenous compounds and xenobiotics. We have previously observed that ABC transporters P-glycoprotein (P-gp) and Multidrug resistance-associated protein 2 (Mrp2) were differentially expressed in kidney and intestine after kidney IR.

Objective: Since P-gp and Mrp2 are also expressed in the liver, the aim of this study was to evaluate if kidney IR injury affects their liver expressions.

Methods: Male Wistar rats were subjected to 40 min of unilateral renal ischemia followed by 24h of reperfusion (IR, n=5). Sham-operated controls (C, n=5) were also processed (Institutional Animal Care and Use Committee, FBIOyF, UNR, ResN°850/2022). Liver function was assessed by AST, ALT and alkaline phosphatase (AP) plasma activity by spectrophotometry. P-gp and Mrp2 expressions in liver homogenates ($P\text{-}gp_{LH}$ and $Mrp2_{LH}$) and total plasma membranes ($P\text{-}gp_{Lm}$ and $Mrp2_{Lm}$) were evaluated by immunoblotting. t-Student *:p<0,05.

Results: (Mean±S.E.M.): After IR-induced kidney injury, liver enzymes, AST and AP, were significantly increased (AST(UI/L): C=35,40±4,60, IR=69,10±0,01*; ALT(UI/L): C=6,25±0,85, IR=7,00±0,08; AP(UI/L): C=100,5±3,9, IR=211,5±27,2*). Concurrently, plasma membrane expression of P-gp and Mrp2 in the liver was significantly decreased ($P\text{-}gp_{LH}$(%): C=100±4, IR=104±3; $P\text{-}gp_{Lm}$(%): C=100±3, IR=89±2*; $Mrp2_{LH}$(%): C=100±3, IR=76±3*; $Mrp2_{Lm}$(%): C=100±3, IR=80±2**.

Conclusions: The decreased liver expression of ABC transporters could have clinical consequences in handling of compounds of pharmacological interest and can contribute to the pathophysiological process triggered by kidney injury. Further studies will be aimed to get insight into the mechanisms involved in liver injury.

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Upregulation of TGF-β1, fibronectin and NOX-4 along with augmented reactive oxygen sepecies in renal medullary tissues from male and female mice during a short period of streptozotocin-(STZ)-induced type-1 diabetes mellitus

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Introduction: Diabetes mellitus (DM) can lead to kidney damage related with increased reactive oxygen species (ROS), proteinuria, and tubular damage. Altered protein expression levels of transforming growth factor-beta 1 (TGF-β1), fibronectin and NADPH-oxidase (NOX-4) are associated with the profibrotic phenotype in renal tubular cells. NOX-4, one of the primary sources of ROS in diabetic kidney and responsible for the induction of profibrotic factors of collecting duct (CD) cells. Currently there is no literature describing the expression of these markers in male and female mice during early DM.

Objetive: Our aim was to evaluate changes in transcript and protein abundance of TGF-β1, fibronectin and NOX-4 along with ROS levels in renal medullary tissuesfrom male and female mice during a short period of streptozotocin-(STZ)-induced type-1 DM.

Methods: CF-1 mice were injected with (n=6) or without (n=6) a single dose of STZ (200 mg/Kg) and euthanized at day 6 fo further analysis of medullary tissue. Results (mean ± SEM) were analyzed using Shapiro-Wilk test, Two-way-ANOVA and non-paired t-test. Animal studies were

in accordance with the Animal Care and the Bioethical Comittee of the PUCV (BIOEPUCV-B 267-2019).

Results: STZ-females showed higher expression of fibronectin and TGF-β1 when compared to control mice of either gender. Interestingly, STZ-female mice showed a >30-fold increase of mRNA levels and a 3-fold increase in protein levels of kidney medullary NOX-4. Both male and female-STZ mice showed increased intrarenal ROS. Early STZ-induced DM elevates profibrotic markers expression in medullary CD cells.

Conclusion: Our results suggest that NOX-4 regulates antioxidants differentry in males and females.

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Characterization of primary cultures of renal inner medullary collecting ducts of human origin

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Introduction: Here, we present a protocol to develop primary cultures of renal inner medullary collecting duct of human origin (hIMCD) suitable for elucidating molecular mechanisms involved in the regulation and function of sodium and water transport along with insights of pathophysiological mechanisms involved in the expression of markers of tubular injury after several stimuli such as aldosterone, angiotensin II, high glucose among others.

Objectives: Obtain hIMCD from human inner medullas for primary cultures.

Methods: Fresh tissue from patients underwent radical nephrectomy at Clínica Dávila (Santiago, Chile) was obtained to separate and mince inner medullary tissues. After enzyme digestion with hyaluronidase and collagenase, the cells were washed and seeded in culture dishes with hyperosmotic media as selective osmolarity conditions. Ethical approval was obtained from the clinic Scientific Ethics Committee (n°01-25-21).

Results: Expression of V2R, AQP2 and ENaC was demonstrated using fluorescence microscopy. ENaC protein expression resulted in 3-fold increase as compared to controls ($p<0.05$, t-test) after 8 h of aldosterone (10 nM) incubation.

Conclusion: With standard laboratory equipment hIMCD cells can be cultured in regular cell culture dishes and used for experiments after seven days at optimal confluency of 70-80 %.

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Study of the A2BAR in profibrotic activation of glomerular parietal epithelial cells in experimental diabetes

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Introduction: Diabetic nephropathy (DN) is a complication of diabetes characterized by the loss of podocytes which results in the development of proteinuria and glomerulosclerosis. Podocytes are terminally differentiated cells without the capacity to selfrenew and while the parietal epithelial cells (PEC) of the Bowman's capsule have been identified as podocyte progenitors, however some studies have shown that in DN, PEC cells undergo profibrotic activation. The progression of DN has been correlated with high levels of adenosine. Because we have determined that the antagonism of adenosine receptor A2B (A2BAR) attenuates fibrosis and proteinuria in experimental DN, this study addresses role of A2BAR on the profibrotic activation of PEC.

Methods: The use of animals for this project was approved by Bioethic Committee nº 465/2022. Experimental diabetes was induced in rats using streptozotocin. Following one month after diabetes induction, the A2BAR antagonist MRS1754 (0.5mg/kg/48h) was administered to

diabetic rats for 4 weeks. The PEC CD24+CD56+ subpopulation and PEC CD9+CD44+αSMA+ cells undergoing profibrotic activation were analyzed through immunohistochemistry and western blot. The statistical analysis utilized was Student *t* test for 3 individual samples.

Results: We determined a decrease in CD24 and CD56 levels and an increase of the profibrotic markers CD9, CD44 and αSMA in the bowman´s capsule of diabetic rats. The use of A2BAR receptor antagonist MRS1754 in diabetic rats attenuates the increase of CD9, CD44 and αSMA and increase the levels of CD56.

Conclusions: The antagonism of A2AR blocks the profibrotic activation of PEC, which may preserve their potential to differentiate toward podocyte.

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Acknowledgments: Acknowledgements to the entire Molecular Pathology Laboratory team.

AREA: SKELETAL MUSCLE AND EXERCISE PHYSIOLOGY

P-69 ★*selected for oral communication*

Fecal microbiota transplant from young-trained donors reduces intestinal permeability in old mice

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Introduction: Increased intestinal permeability in aging is closely associated with age-related diseases. This heightened permeability allows substances like LPS and others to enter the bloodstream, contributing to chronic inflammation during old age, often termed "inflammaging." Chronic exercise can improve gut microbial composition and promote gut health. Therefore, interventions targeting the microbiota, such as Fecal Microbiota Transplantation (FMT), hold promise for modulating permeability and promoting healthy aging.

Objective: To investigate the impact of FMT from young-trained donors on gut permeability in elderly mice.

Methods: C57BL/6 elderly male mice (18 months old) received FMT from young (4 months) trained donors. Control aged group recieved its own FMT. Young animals were trained for 6 weeks with a treadmill protocol (1 hour daily, 5 days per week, at a speed of 12 m/min). A sedentary group was selected as control. To assess intestinal permeability, we used a quantitative fluorometric method with FITC-dextran. CICUAL Universidad de Valparaíso approved experimental procedures (BEA179-22).

Results: A chronic exercise routine reduces colonic permeability in young mice (N=5-6 per group; $p<0.05$, mean ± SEM, Mann-Whitney test), without affecting ileal permeability. In aged animals (18 months) that received FMT from young-trained donors, we observed a similar behavior, a decrease in intestinal permeability in the colon (N= 8 per group; $p<0.05$, mean ± SEM, Mann-Whitney test), with no effects in the ileal region.

Conclusion: Our data suggests that chronic exercise can reduce colonic permeability through modulation of the intestinal microbiota, and that FMT from young-trained donors is able to transmit this effect to aged recipients.

Financing: FONDECYT 1122097 and NAM21I0063.

P-70

Evaluation of the administration of a isolate vegetable protein based on quinoa and lentils of high nutritional quality to delay the development of sarcopenia associated with aging

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Introduction: Sarcopenia is a condition characterized by the loss of muscle mass and strength. While this condition is associated with aging, it can also develop as a result of physical inactivity or inadequate protein intake.

Objetive: To assess the impact of a protein isolate made from quinoa and lentils, known for its high nutritional quality, in preventing age-related sarcopenia.

Methods: A protein isolate from quinoa and lentils was prepared in a 1:9 ratio. Nineteen male C57BL/6 mice were used, obtained from the Chilean Public Health Institute, with the use of experimental animals approved by the Institutional Ethics Committee of the University of Santiago de Chile for the dicyt associative project code: 022087CB_DAS. The mice were divided into young control, elderly control, and elderly supplemented groups. Supplementation involved 2 mg of protein per kg of body weight for 14 weeks, and the onset of sarcopenia was determined through tests involving inverted grip, weight lifting, and running at 80% of maximum speed. Data were analyzed using two-way ANOVA and post hoc Holm-Sidak tests.

Results: Preliminary results at 6 weeks were not significant ($p>0.05$). Initial data from the inverted grip test showed a difference of 18.62 with an SD of ± 4.3.

Conclusion: While preliminary results are not significant, the methodology for protein isolate administration was standardized, and initial data suggest promising effects in the inverted grip test. These findings support the continuation of the study to thoroughly assess the impact of the protein isolate in preventing age-related sarcopenia.

Financing: This research was carried out and financed within the framework of the associative Dicyt project 022087CB_DAS of the Universidad de Santiago de Chile.

Acknowledgments: to the team of the reproductive immunology laboratoryTo professor Patricia Reuquen Lopez.

P-71

Increased intermuscular fibro-fatty tissue and reduced muscle quality as traits of an accelerated ageing phenotype in young adults

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Introduction: Proper function and quality of muscle support metabolic homeostasis and physical performance. Intermuscular fibro-fatty infiltration (IMFFAT) and sarcopenia impair function, increase cardiometabolic risk and disability, characteristics of unhealthy ageing. Whether IMFFAT and poor muscle quality at young ages denote accelerated muscle ageing is unknown.

Methods: We tested this hypothesis in 13 lean subjects (2 female; 29.4±0.7y, BMI: 23.6±3.3) from the Santiago Longitudinal Cohort. Historic cardiometabolic and BMI changes were retrospectively analyzed. Cross-sectional muscle morphology and function were assessed (MRI; BIA); isometric knee extensor strength ($MVKE_{iso}$), handgrip, rectus femoralis thickness and pennation, VO_{2max}, and muscle oxygenation. Ethical permission granted by a certified IRB (12/2021;31-21).

Results: BMI trajectory (0-to-22y) correlated positively with BMI_30y ($r=0.68$, $p=0.02$). Subjects with history of greater BMI gain had greater HGS, $MVKE_{iso}$ and torque (ES: 1.03, 0.44, 0.42) despite more adiposity (muscle-to-fat ratio, ES: -0.53). Subjects with high $IMFFAT_{30y}$ had higher SBP, insulin, HOMA-IR and HDL-c (ES: -0.35; -0.42; -0.38; -1.21). Metabolic profile 16-to-30y worsened in those with higher IMFFAT. IMFFAT correlated positively with BMI_30y, waist-to-height ratio, fat masses, and negatively with thigh muscle CSA ([r, p] = 0.6, 0.03; 0.7, 0.008; 0.70, 0.008; 0.79, 0.001; 0.65, 0.017; 0.69, 0.01; -0.63, 0.022). $IMFFAT_{30y}$ correlated negatively with $MVKE_{iso}$, torque, VO_{2max}, VT_1 and HR_{max} ([r,p] = -0.53, 0.05; -0.58, 0.03; -0.55, 0.05; -0.56, 0.04).

Conclusion: In young, lean individuals, poor muscle function and quality associated with increased IMFFAT levels, which impairs metabolic profile and cardiorespiratory fitness. Even in young adults, this may indicate an accelerated state of muscle ageing

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P-72

Resistance exercise improves physical abilities of subjects exposed prenatally to ethanol

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Introduction: Ethanol consumption during pregnancy prompts fetal alcohol spectrum disorder (FASD) with cognitive as well as motor disabilities. The prevalence of FASD reaches 1% worldwide. Unfortunately, there is no effective treatment. Exercise is a promising and easily accessible treatment option for mental and motor alterations. It has been described that physical exercise releases irisin, a myokine promoting neuroplasticity and cognitive improvement.

Objective: We aim to study whether resistance exercise (RT) interventions improve the motor disabilities of FASD animals model by increasing plasmatic irisin levels.

Methods: Sixteen Sprague-Dawley rats were divided into 4 groups: control (CTRL) and FASD trained or not with RT for six weeks. The muscle strength (MS) was measured by time hanging on a mesh (THM), in all groups before (B) and after (A) RT intervention. Plasmatic irisin levels were measured after an exercise challenge. The project was approved by the CEC-UCN N° 39/2022.

Results: The RT increases THM only in FASD (221.4±28.87 s; 62.86±5.75 s, B vs A; $p<0.0001$; ANOVA and Tukey Test) and not in CTRL. No differences in plasmatic Irisin levels were found in any group (24.7±11.9; 14.7±1.9; 8.7±0.97; 11.5±1.2 ng/ml, mean ± SEM, $p>0.05$ ANOVA and Tukey Test).

Conclusion: Ethanol consumption during pregnancy affects motor skills in adolescent offspring. The RT intervention was effective in improving the MS in FASD subjects. Even though more experiments are needed to understand the molecular mechanism, our results support using evidence-based exercise intervention to prescribe in FASD subjects clinically.

Financing: Proyecto Semilla 2022 N°666 of the Universidad Católica del Norte (UCN).

P-73 ★*selected for oral communication*

The structure, stability, and function of the neuromuscular synapse is altered upon activation of the canonical Wnt signaling pathway

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Introduction: Motor axon terminals, skeletal muscle fibers, and capping terminal Schwann cells (tSCs) assemble neuromuscular junctions (NMJ). Although canonical Wnt/beta-catenin signaling becomes activate in conditions affecting the NMJ, its consequences on NMJ integrity and function are unknown.

Objective: We analyzed NMJ structure, stability, and function upon modulating a Wnt ligand, a Fzd receptor, and a key intracellular effector of Wnt/beta-catenin signaling.

Methods: NMJ morphology was quantified by NMJ-morph; NMJ stability by pulse/chase experiments using alpha-bungarotoxin; NMJ function by electrophysiology and electromyography. Samples were obtained from: (i) mice electroporated in vivo to overexpress Wnt3a, or (ii) the Fzd9 receptor. NMJ stability and function was also studied in (iii) denervated mice treated with the GSK3beta inhibitor Andrographolide. Data are expressed as mean +/- SEM of N=3. Significance was evaluated by t-test or ANOVA. Procedures were approved by the Institutional Bioethical Committee (UACh C97-2023).

Results: Muscle Wnt3a overexpression resulted in progressive NMJ postsynaptic fragmentation and altered expression of AChR subunits and rapsyn. Also, we found partial denervation, thinner axons, and aberrant tSCs distribution. Pharmacological Wnt activation after nerve damage resulted in bigger postsynaptic domains, axonal thinning, impaired reinnervation, and tSCs loss from NMJs. Wnt3a and Fzd9 overexpression resulted in reduced AChR turnover in the postsynaptic domain. Remarkably, while Wnt3a overexpression resulted in higher neurotransmission, Fzd9 overexpression and pharmacological Wnt signaling activation impaired functional nerve-muscle communication.

Conclusion: Canonical Wnt activation induces aberrant remodeling of NMJ postsynaptic components, alters its stability, and induces changes in presynaptic terminals and tSCs. However, neurotransmission was differentially affected by Wnt activation.
Financing: Funded by FONDECYT 1221213 to JPH.

P-74

Incidence of muscular strength, anthropometry, and asymmetry in the general index of autonomy in older chilean women

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Introduction: Frailty affects the functional autonomy (FA) of the elderly and could manifest itself in muscle imbalances in the extremities, resulting in a disparity in size and strength between them. One contributing factor to this phenomenon is the dominance of one limb over the other. In Chile, information on the relationship between muscle strength (MS) levels and FA asymmetries in older women is limited.

Objective: This study related the levels of MS, anthropometric parameters, and asymmetries of the lower and upper extremities, with the FA of a group of older Chilean women.

Methods: 39 women aged 71.3±1.36 years participated, and their FA was evaluated using the GDLAM index (IG). Based on the score obtained in the IG, they were classified by percentiles as Group 1 (G1; n=20) with favorable FA (P≤50) and Group 2 (G2; n=19) with low FA (P>50). The study and the informed consent were approved by the Scientific-Ethical Committee of the Universidad de Las Américas, Chile (CEC_PI_2023003).

Anthropometric parameters were BMI, fat percentage, bone mass, circumferences (arm, thigh, calf), diameters (humerus, femur) and upper/lower extremity strength was evaluated to determine asymmetries. The differences between the covariates of both groups were evaluated using the student's t test and the Mann-Whitney test for independent samples.

Results: G1 presented less asymmetry ($p<0.05$) in the lower extremities and greater calf circumference than G2 ($p<0.05$). G1 presented greater bilateral strength (dominant and non-dominant limb) compared to G2 ($p<0.05$).

Conclusion: The covariates of age, anthropometry, MS, and lower/upper extremity asymmetries influence FA in older women.

Financing: This research was not funded.

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P-75

Energy expenditure and substrate oxidation during and after an eccentric, concentric and mixed resistance exercise session in sedentary men

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Introduction: Nowadays, muscular resistance exercise (RE) is essential for health (Saeidifard et al., 2019) and body composition (El-Kotob et al., 2020). However, differences in energy expenditure (EE) and substrate oxidation depending on the type of muscle contraction in adults are not completely defined.

Objective: To determine EE and substrate oxidation (carbohydrates (CHO) and fats (FAT) at rest, during, and up to 1 hour after eccentric (ECC), concentric (CON), and mixed (MIX) muscle strengthening.

Methods: Sedentary men (n=7) (mean ± standard deviation (SD)), age = 25.0 ± 5.7 years, and BMI 25.2 ± 1.0 kg/m^2 were recruited. Indirect calorimetry was evaluated during (DUR), immediately after (INM_post), and 1 h after (1h_post) RE protocol performed at 75% of 1 repetition maximum to estimate FAT and CHO oxidation. This project was approved by the Bioethics Committee of Universidad Andrés Bello (approval record 030/2022). A variance test

(ANOVA) was performed with Bonferroni post hoc analysis, considering a value of $p < 0.05$ as statistically significant.

Results: DUR, similar FAT oxidation was found between muscle contraction types, but greater CHO oxidation was found during ECC ($p=0.01$). INM_post-exercise CHO oxidation was similar between exercises, but MIX showed significantly greater FAT oxidation ($p=0.04$) than CONC and ECC. No significant differences were found between groups in CHO and FAT oxidation.

Conclusion: The results highlight the importance of selecting the type of muscle contraction when applying RE due to the responses associated with EE and substrate oxidation, which could be helpful in the management of some health conditions or body composition.

Financing: Proyectos Ciencias Biomédicas y Clínicas N° DI-05-CBC/22.

P-76

Human fiber-specific muscle differences in protein synthesis and degradation pathways, and oxidative metabolism between obese and healthy individuals

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Introduction: Obesity has been shown to induce muscle atrophy. However, how specific muscle fiber adapt to obesity has not been investigated.

Objective: to compare protein levels of the protein synthesis/degradation pathways and oxidative metabolism-related proteins in type I and IIa muscle fibers and whole muscle homogenates from obese and healthy individuals.

Methods: Nine obese and nine healthy individuals' muscle biopsies were analyzed to identify type I and IIa MHC isoforms. Akt-1, mTOR, p70s6K, S6 ribosomal protein, ERK1/2, p38, COX-IV, DRP1, Mitofusin-2, AMPK total proteins and Mitochondrial Oxidative Phosphorylation System (OXPHOS) from whole muscle and specific fiber-type were quantified. Institutional ethical approval (#032/2021). Student´s t-test and two-way ANOVA were used to compare obese and healthy groups for whole homogenated and specific fibers.

Results: Akt-1, mTOR, p70S6K, and S6RP protein levels were similar between muscle fiber types and whole muscle from obese and healthy individuals. COX-IV was similar between groups, showing greater levels of COX-IV in type I fibers. Obese showed greater Complex II, IV, and V levels in type I than IIa. Obese showed similar DRP-1, total ERK, and AMPK protein levels in both fiber types. Healthy showed greater DRP-1 levels in type I than IIa fibers. Healthy showed greater ERK and AMPK protein levels in type IIa than in type I fibers. Total ubiquitination was similar between fiber types and whole muscle.

Conclusion: These results suggest that healthy individuals have more efficient protein synthesis machinery in type IIa fibers, mainly through MAP-Kinase. Differences in OXPHOS suggest differences in oxidative activity between groups.

Financing: This project was funded by FONDECYT #1211962 project.

Thursday 30

AREA: EDUCATION

P-1 ★*selected for oral communication*

Development of a thermostatic temperature control system to teach concepts on physiological regulation

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Introduction: In some textbooks homeostatic regulation is Illustrated by a thermostatic temperature control system, such as thermoregulated water bath. The operation of such a system may not be familiar to many students, and the proposed analogy may not be fully understood.

Objective: To overcome this problem, we have built a temperature-controlled system to be used during a comparative animal physiology course for Biology students of a Brazilian public university.

Methods: The system consists of a temperature controller connected to a temperature sensor, two heating (light bulbs) and two cooling (fans) elements, with exposed electrical wiring; one lamp and one fan are connected to a dimmer switch, allowing for their power control. A bedside lamp and an artificial ice pack are used as external heating and cooling elements.

Results: The system depicts all the components of a feedback loop, simulating either physiological or behavioral thermoregulation in a real-time process. The system also enables the students to examine three of Cannon's postulates (the role of the nervous system in maintaining homeostasis and the concepts of tonic and antagonistic control). The system may also be set to simulate fever (both in endothermic and ectothermic animals) as an example of allostatic regulation, and to depict hyperthermia and hypometabolic states.

Conclusions: We have not yet conducted a quantitative assessment of the system effectiveness in enhancing student learning. Initial data based on informal qualitative assessment, however, indicate that it is a useful educational resource for teaching concepts on physiological regulation.

Financing: None

P-2 ★*selected for oral communication*

The identification of behavioral patterns seems to improve e-learning of integrative physiology through core concepts

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Introduction: The COVID-19 pandemic forced a sudden shift to virtual education worldwide in 2020, affecting even societies with limited resources.

Objective: Identify and develop activities to manage negative behavioral patterns generated by the study of integrative physiology by using an adaptation of the behavior change wheel.

Methods: 34 students enrolled in the integrative physiology course for 2nd-year medical students (fall 2021) were organized into 4 groups. Each group developed academic content using the core concepts together with an instructor. The strategy revolved around recognizing emotions and categorizing it as behavioral patterns (NA non-adequate: when negative emotions were present >50% of participants., and A-adequate: when negative emotions were present < 50% of participants) during each virtual session (total virtual sessions=6). Finally, each session ended by brainstorming activities to address NA behavioral patterns. This teaching tool was approved by the Academy of Clinical and basic sciences and the curricular commission belonging to the Bachelor of Medicine of the FEPZH-UASLP, México.

Results: 80% of the students found the combination of this technique to be beneficial, as it provided a structured approach to identify and manage negative emotions associated with complex topics.

Conclusion: this study has shown promise in using the behavior change wheel in conjunction with core physiological concepts to help students navigate their emotions. The next step involves

refining and implementing more fully characterized interventions based on the measured results.

Financing: Autonomous University of San Luis Potosí, México, C20-FAI-10-56.56

AREA:GENERAL

P-3

Reactive hyperemia and cardiovascular autonomic neuropathy in type 2 diabetic patients: A systematic review of randomized and nonrandomized clinical trials

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Introduction and Objective: This work aimed to determine the relationship between the autonomic nervous system and reactive hyperemia (RH) in type 2 diabetes patients.

Methods: A systematic review of randomized and nonrandomized clinical studies characterizing reactive hyperemia and autonomic activity in type 2 diabetes patients was performed.

Results: Five articles showed differences in RH between healthy subjects and diabetic patients, while one study did not show such differences between healthy subjects and diabetic patients, but patients with diabetic ulcers had lower RH index values compared to healthy controls. Another study found no significant difference in blood flow after a muscle strain that induced reactive hyperemia between normal subjects and non-smoking diabetic patients. Four studies measured reactive hyperemia using peripheral arterial tonometry (PAT); only two found a significantly lower endothelial-function-derived measure of PAT in diabetic patients than in those without cardiovascular autonomic neuropathy (CAN). Four studies measured reactive hyperemia using flow-mediated dilation (FMD), but no significant differences were reported between diabetic patients with and without CAN. Two studies measured RH using laser Doppler techniques; one of them found significant differences in the blood flow of calf skin after stretching between diabetic non-smokers and smokers.

Conclusion: Blood flow alterations in diabetic patients during RH are mainly mediated by sympathetic dysfunction. The greatest evidence suggests a relationship between ANS and RH; however, there are no significant differences in RH between diabetic patients with and without CAN, as measured using FMD. When the flow of the microvascular territory is measured, the differences between diabetics with and without CAN become evident. Therefore, RH measured using PAT may reflect diabetic neuropathic changes with greater sensitivity compared to FMD.

P-4

Anomalous behavior of cell membrane under electric stress

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Introduction: Cell membrane at high physiological and supraphysiological voltages (subjected to electrostimulation, defibrillation, pulsed field ablation) can lose its barrier function (electroporation) and change its response to the electric field, with numerous consequences for cell function and survival. Biophysical details of electropore formation and membrane conductance at high transmembrane potentials (TMP) are not understood.

Objective: Visualize electropore formation and dynamics, measure single pore conductance and longevity, and explore the adaptive change in membrane conductance.

Methods: We used whole-cell patch clamp and total internal reflection fluorescence (TIRF) on an electroconductive indium tin oxide substrate to visualize Ca^{2+} puffs through individual pores concurrently with current measurements.

Results: Transient membrane disruptions resulting in diffuse regional Ca^{2+} entry were recorded at high physiological TMP of (-100) – (-120) mV. Hyperpolarization down to (-200) – (-250 mV) caused formation of distinct focal pores with single pore conductance from tens of pS to nS. We observed both transient electropores which closed immediately after the electric pulse, and persistent pores which remained open for up to a minute. At about (-250) mV, cell membrane adaptively increased conductance in response to larger hyperpolarizing steps. Cells effectively "clamped" the TMP at about (-250) mV and no further hyperpolarization could be reached regardless of the command voltage applied.
Conclusion: For the first time, we discerned individual electroporation lesions in live cells. We proved that discrete focal pores can stay open for tens of seconds after an electric shock and established the adaptive membrane conductance phenomenon near the TMP ceiling.
Financing: Supported in part by NIH NEI R21EY034258 and R21EY034803.

P-5

Stress-induced cell senescence increases calcium transfer between the endoplasmic reticulum and mitochondria

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Introduction: Cell senescence and mitochondrial dysfunction are intertwined. Mitochondrial Ca2+ overload from endoplasmic reticulum (ER) stores induces mitochondrial dysfunction; however, it is unknown whether it participates in mitochondrial dysfunction during cell senescence.
Objective: Here, we aimed to determine whether changes in ER-mitochondria Ca2+ transfer mediates cell senescence induced by oxidative stress in HepG2 cells.
Methods: Cell senescence was induced by oxygen peroxide exposure for 1 h. After 24-72 h, we evaluated senescence as: β-galactosidase activity (n=3), and protein expression of p16, p21 and p53 (n=3). ER-mitochondria Ca2+ transfer was evaluated with Cepia2mt in live cells (fluorescence microscopy, n=3-4), mitochondrial respiration (n=4-6), ROS production (flow cytometry, n=4), mitochondrial transmembrane potential (immunofluorescence, n=4), and OXPHOS protein expression (n=4). To modulate Ca2+ signaling, we used 2-APB and Ru360 (inhibitors of ER-mitochondria Ca2+ transfer), and BAPTA (cytosolic Ca2+ chelator). Cell viability was measured through MTT (n=4). Data are expressed as mean ± SEM, and were analyzed through one-way ANOVA, or two-way ANOVA when an inhibitor/chelator was used.
Results: After treatment, cells developed cell senescence as well as increased ER-mitochondria Ca2+ transfer. To assess whether this increase mediates cell senescence, we inhibited it with 2-APB, or Ru360, both of which failed to prevent the loss of viability measured through MTT assay. Instead, BAPTA rescued cell viability, indicating that intracellular Ca2+ does participate in the development of cell senescence.
Conclusion: During oxidative stress, ER-mitochondria communication increases, which is apparently not required for the development of cell senescence. Rather, it is cytosolic Ca2+ that mediates this process.
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P-6

The Ca2+-activated chloride channel in ATP-induced contraction in small intrapulmonary

vessels from ex vivo precision cut lung slices
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Introduction: ATP contracted of Small Intrapulmonary Vessels (SIVs) via of purinergic receptors (Henríquez M, et al., J Physiol, 2018, DOI: 10.1113/JP274731). Surprisingly, ATP-induced vasoconstriction is inhibited by dihydropyridines which specifically block the Cav 1.2 mediated vasoconstriction. TMEM16A is described in smooth muscle cells. We postulate that part of ATP-induced contraction is mediated by activation of TMEN16A, membrane depolarization and Cav 1.2-induced vasoconstriction.

Objective: To evaluate the participation of the TMEM16A in ATP-induced contraction of SIVs using *ex vivo* precision cut lung slices (PCLS).

Methods: PCLS from Sprague-Dawley (~340g) and phase contrast videomicroscopy were used to quantify SIVs contraction in response to ATP (n=15) with or without Ca^{2+} (n=7) and NaCl (n=15) or NaI (n=9) and also the inhibition experiment using TMEM16A inhibitors: Ani9 (n=7), T16AinhAO1 (n=4) and Benzbromarone (n=7) (Protocol CBA1183 FMUCH). All results are shown as median and interquartile range. We used non-parametric Kruskal-Wallis and Wilcoxon tests and $p<0.05$ to be considered significant.

Results: The 100 µM ATP with 0 mM Ca^{2+} showed contraction of 81.1% (95.9%-73.1%) then a fast relaxation at the end of ATP stimulation reached only 44.7%. Similar results were obtained replacing the NaCl by NaI. Vasoconstriction with 100 µM ATP with NaI, the kinetic of contractions were slower than the observed in NaCl. Vasorelaxing effect of TMEN16A inhibitors on the ATP-induced vasoconstriction was also observed showing a decrease by 43.3%, 42.4% and 9.2% using ANI9, T16inhAO1 and Benzbromarone, respectively.

Conclusion: TMEM16A is functional in SIVs smooth muscle cells and participates in ATP-induced muscle contraction.

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P-7

Opsin 3 in T Lymphocytes: Temporal modulation and its relationship to the circadian molecular
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Introduction: Opsin 3 (OPN-3) is a photoreceptor whose function remains unknown, although it is prevalent in most of mammalian tissues, including lymphocytes. Given its photoreceptive capability and the impact of circadian clock genes on lymphocyte migration [Druzd et al., 2018], we proposed that OPN-3 can be implicated in the integration of circadian rhythms and immune system migration.

Objectives: To ascertain the circadian expression of OPN-3 in murine T lymphocytes (LT) and its correlation with core circadian clock genes (Bmal, Per2, Per3), as well as genes involved in lymphocyte migration (CCR7) we will characterize OPN-3 expression in murine LT under stable entrainment to a 12:12 light-dark cycle through RT-PCR and Western blot.

Methods: Spleen samples were obtained from mice (n=24) (Bioethics approved protocol number: 1196) entrained to a 12:12 light-dark cycle at different times. RT-PCR analyses were conducted for core circadian clock genes (Bmal, Per2), lymphocyte migration gene (CCR7), and OPN-3. Statistically significant differences were assessed using a one-way ANOVA followed by a correction Geisser-Greenhouse. All data are represented as mean ± SEM. Additionally, protein expression of OPN-3 was analyzed via Western blot.

Results: Significant differences were observed in the gene expression of OPN-3 throughout the day, with lower gene expression at the onset of the subjective day.

Conclusion: increased gene expression at the onset of the subjective night, suggesting a potential link to the amount of light received by the organism and lymphocyte migration processes.

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P-8

Obesity is associated with premature aging in young adults from a Chilean birth cohort: preliminary results from the ObAGE Study

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Introduction: Obesity has been found to contribute to the onset of premature aging. Theories suggest that obesity may accelerate normal aging, leading to decreased longevity and quality of life, the mechanisms behind this phenomenon are not yet fully understood.
Objective: To investigate the link between early-life obesity exposure and accelerated aging.
Methods: Multiple-events case-control study in a Chilean birth cohort. In n=205 30-year-old body mass index (BMI) was estimated since birth. Three trajectories were traced: always healthy BMI (AH:29.1±0.4y), adolescent obesity (AdOB:28.7±0.4y), and childhood obesity (ChOB:28.8±0.4y). Participants underwent anthropometric, DXA, and liver function assessment; Horvath's epigenetic clock (DNA methylation age calculator, DNAmAge) was determined in peripheral blood mononuclear cells (PBMCs) Ethical approval was obtained by the Scientific Ethics Committee of INTA (12-2021; Nº21–037).
Results: Although chronological age was the same in all groups, DNAmAge was higher in AdOB (33.1±3.7y) and ChOB (33.5±4.3y) than AH (28.5±2.5y). The effect of BMI trajectory on DNAmAge was significant and large in both males and females (Cohen's f of 0.77 and 0.56, respectively). Several anthropometric and body composition markers predict DNAmAge, with stronger correlations observed in males/females. There was a strong positive relationship between DNAmAge and several cardiometabolic markers. Cardiometabolic parameters remained significant in both sexes. However, the metabolic syndrome severity score and glucose homeostasis only remained significant in males.
Conclusions: Our research suggests that early-life obesity exposure can increase DNAmAge, accelerating aging in young adults. Similarly, a higher DNAmAge seems to be associated with an increased risk of cardiometabolic problems in young adults.
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P-9

Expression of calcium-dependent potassium channels (KCa) in placenta in fetoplacental unit of gestational diabetic patients

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Introduction: Gestational diabetes is a pathology characterized by endothelial dysfunction, associated with potential alteration of endothelium-dependent hyperpolarization (EDH). There is evidence that in gestational diabetes there is higher endothelial hyperpolarization, but Calcium-dependent potassium channels (KCa) have an important role in EDH, so its expression and function could be altered by gestational diabetes.
Objective: To compare the expression of KCa

channels in gestational diabetes patients and healthy controls.

Methods: Total RNA was extracted from placenta and human umbilical vein endothelial cells (HUVECs) of gestational diabetes (n=17) and healthy control (n=10) (Ethics committee certification: CEC-SSC 21-04-24). RT-PCR was made using primers for large conductance (BKCa) and intermediate conductance (IKCa) potassium channels and 28S mRNA. Results were visualized by agarose gel and analyzed with Image J software. Protein expression of α subunit of BKCa was evaluated by immunofluorescence in HUVECs. Values are mean ± SEM, analyzed by non-parametric U Mann-Whitney test.

Results: The relations BKCa/28S and IKCa/28S were statistically equal in chorionic plate samples from gestational diabetes compared with healthy controls (p=0.64 and p=0.37, respectively). In gestational diabetes HUVECs, there is a non-significant (p=0.19) tendency to decrease BKCa mRNA levels, but the immunofluorescence showed higher expression of BKCa protein in gestational diabetes.

Conclusions: In the fetoplacental unit, gestational diabetes could induce high expression of BKCa α-subunit, through a non-transcriptional mechanism. The post-transcriptional mechanisms induced by gestational diabetes could be related to micro RNA 222, which has been shown to be highly expressed in the fetoplacental endothelium of gestational diabetes.

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P-10

Gestational COVID-19 increases the expression of calcium-activated potassium channels (KCa) in human placenta

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Introduction: During pregnancy, SARS-CoV-2 infection and COVID-19 pandemic increased the risk of preterm birth and other obstetrical adverse outcomes. In gestational COVID-19 cases, placental vascular injury and thrombosis have been reported. The calcium-activated potassium channels (KCa) are relevant for placental function, but there is no information about gestational COVID-19 and KCa expression.

Objective: To determinate the expression of KCa channels in placental tissue from COVID-19 cases.

Methods: RNA extraction (Total RNA mini kit, Geneaid) was performed from chorionic plate and chorionic villi of placenta donated by COVID-19 cases (n=11) and healthy controls (n=6) (Ethics committee certification: CEC-SSC 21-04-24). RT-PCR for KCa and 28S genes and agarose gel electrophoresis was made. Semiquantitative analysis of the gels was performed using Image J software. The KCa/28S relation was analyzed using the GraphPad Prism software and non-parametric U Mann-Whitney test was applied. Values are mean±SEM.

Results: In the chorionic plate of COVID-19 patients, there was a 3.9±0.4-fold increase of large conductance KCa (BKCa) mRNA, with especially marked increase in severe COVID-19 patients. There were no differences of BKCa expression in chorionic villi. In the case of intermediate conductance KCa (IKCa), there is an increase of 2.3±0.4-fold in the mRNA levels in chorionic villi of COVID-19 patients, especially in patients mild COVID-19.

Conclusion: The gestational COVID-19 seems to increase the expression of BKCa in chorionic plate and IKCa in chorionic villi of placenta. This effect could be linked with a mechanism of compensatory vasodilation carried out by the placenta, in response to the vascular injury.

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AREA: CARDIOVASCULAR AND RESPIRATORY

P-11

Treatment with nonsteroidal anti-inflammatory drugs affect endothelial cells signaling

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Introduction: Prostanoid production through cyclooxygenase (COX) is involved in the progress of inflammation. Consequently, COX blockade by nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and diclofenac, are widely used in the treatment of pain and fever. However, several cardiovascular side effects associated with these drugs have recently been described, which has raised concerns across NSAID therapy.

Objective: To analyze the effect of ibuprofen and diclofenac on the expression and regulation of key signaling proteins for endothelial cell function, including endothelial nitric oxide synthase (eNOS) and voltage-dependent Na^+ channels (Na_v).

Methods: Changes in expression of eNOS and Na_v channel isoforms $Na_v1.2$, $Na_v1.5$ and $Na_v1.6$ were evaluated by Western blot and immunofluorescence in primary cultures of mesenteric endothelial cells. Phosphorylation of eNOS at serine 1177 ($P\text{-}eNOS^{S1177)}$ and at threonine 495 ($P\text{-}eNOS^{T495)}$ was also assessed. This study was approved by the Institutional Bioethics Committee (Protocol #210422002). Data were analyzed by Dunnet t-test (Mean±SEM, n=5) .

Results: The presence of eNOS, $Na_v1.2$. $Na_v1.5$ and $Na_v1.6$ was detected in primary cultures of endothelial cells by immunofluorescence analysis and treatment of these cells for 24 h with 100 µM ibuprofen, but not 100 µM diclofenac, evoked an increase in $Na_v1.5$ channel expression. The presence or subcellular distribution of $Na_v1.2$ and $Na_v1.6$ was not affected by ibuprofen or diclofenac. In contrast, only diclofenac altered eNOS regulation. Application of this drug reduced eNOS expression and $P\text{-}eNOS^{S1177}$ levels, but did not altered $P\text{-}eNOS^{th495}$.

Conclusion: These results indicate that ibuprofen and diclofenac have different side effects that may importantly affect endothelial cell function.

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P-12

Letrozole as a strategy to reduce estrogens in developing a preclinical model of Heart Failure with Preserved Ejection Fraction (HFpEF) in female mice

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Introduction: Estrogens have a protective role in cardiovascular diseases. The preclinical "two-hit" model of HFpEF in female mice generated mild diastolic dysfunction, but it remains unknown whether the differences between males and females are due to these hormones. In order to determine if estrogens are key in the differences in HFpEF phenotype, we used letrozole (aromatase inhibitor) to reduce estrogen synthesis in female mice.

Objective: To evaluate the development of HFpEF characteristics in the two-hit model in female mice with lower estrogen synthesis.

Methods: A subcutaneous letrozole pellet (50 ug/day) was inserted into 5-week-old female C57BL/6N mice (Bioethics Protocol 21454-CQF-UCH), and after 7 weeks they were randomly assigned to 2 experimental groups: LET-Chow and LET-HFD+LNAME 1.5 g/L for 15 weeks. Blood pressure, systolic and diastolic function, exercise, and glucose tolerance were measured at 7 and 15 weeks. Cardiac hypertrophy was determined by HW/TL ratio. Mean±SD, n=5-12 animals per group. Statistical analysis: non-parametric one-way ANOVA. $p<0.05$ was considered statistically significant.

Results: LET-HFD+LNAME mice gained more body weight and showed a decrease in systolic and diastolic pressure compared to the HFD+LNAME group. Exercise intolerance and E/e' did not change between groups fed with HFD but the LET-Chow group showed a mild tendency to increase E/e' ratio compared to the CTRL group. Both groups HFD+LNAME and LET-HFD+LNAME showed glucose intolerance. LET did not change HW/TL, but LET animals were bigger than those without LET (measured by tibia length).

Conclusion: Letrozole modifies HFpEF phenotype in female mice, resembling that observed in males

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P-13

Voltage-dependent Na+ channels play a central role in the control of endothelial cell function in resistance arteries

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Introduction: Endothelium-dependent vasodilator responses are rapidly conducted several millimeters along the length of resistance arteries without apparent decay. The lack of decay indicates that the activation of a regenerative mechanism mediated by voltage-sensitive ion channels, such as voltage-dependent Na+ channels (Na v), may be involved in the response. However, the participation of these channels in the endothelium-dependent vasodilation has not been determined.
Objective: To analyze the participation of Na v channels in the endothelium-dependent vasodilation activated by acetylcholine (ACh).
Methods: The endothelium-dependent vasodilation induced by ACh and Ca2+ waves triggered by single cell mechanical stimulation were evaluated in isolated arterial mesenteric beds of rat and primary cultures of mesenteric endothelial cells, respectively. All studies were approved by the Institutional Bioethics Committee (Protocol #210422002). Data were analyzed by Student's paired t-test or by one-way ANOVA. (Mean ± SEM; n = 6).
Results: The ACh-induced vasodilation was inhibited by the treatment with saxitoxin (300 nM) or the local anesthetic bupivacaine (500 µM). In addition, both the ACh-elicited vasodilation and the single cell mechanical stimulation-activated Ca2+ waves were blocked by tetrodotoxin (TTX) in a concentration-dependent manner (1 µM-100 µM). Consistent with this, the propagation of Ca2+ waves in endothelial cells was also inhibited by bupivacaine and the Na v 1.5-specific inhibitor, Jingzhaotoxin-III (JZTX–III, 1 µM). The expression of the Nav channels isoforms Nav1.2, Nav1.5 and Nav1.6 was confirmed by immunofluorescence and Western blot in primary cultures of endothelial cells.
Conclusion: These results indicate that the activation of Na v channels, most likely the Na v 1.5 isoform, contributes to the vasodilation initiated by endothelium-dependent vasodilators.
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P-14

Purinergic receptor expression in an ex vivo murine model of cigarette smoke exposure

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is prevalent and associated to tobacco, but specific treatment is not available. Precision lung slices (PCLS) can be used as a model of COPD to study signaling, such as purinergic pathway. Only one study described PCLS exposed to cigarette smoke extract (CSE), however validation studies are required.
Objective: To determine purinergic receptor expression in an *ex vivo* model of PCLS exposed to CS.
Methods: PCLS from 1 A/J mice, female, 10 weeks old, were exposed to CSE prepared in serum-free DMEM-F12 using 3 commercial cigarettes, following a protocol approved by Bioethics Committee, University of Chile (CBA1262). Standardization of CSE concentration (0, 0.25, 0.5, 1, 2, 5, 10, 15, 20, 100%) and exposure time (6, 12, 24, 48 h) was performed by determination of cell viability, pH and tar-content. qRT-PCR in PCLS exposed to 10% CSE for 24 h was performed using P2Y6 primers. Nonparametric analysis was used (n=3).
Results: A decrease in viability at 6 h (34.3% viability) and 48 h (76.9% viability) in serum and CSE-free medium was observed. A decrease in viability using 20% CSE 12 h (53.5% viability), and 15% CSE 24 h (61.9% viability) was observed. 100%

CSE pH was 7.35, constant among dilutions, and tar was increased with higher concentrations, with low deviation within replicates. A 2.9 fold-increase in P2Y6 mRNA levels was observed in CSE *versus* control (p>0.05).

Conclusion: This *ex vivo* model of exposure could be useful to study aspects of COPD after acute exposure to CSE.

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P-15

Pharmacological effect of angiotensin-(1-9) in high-fat diet-induced insulin desensitization

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Introduction: Diabetic cardiomyopathy is a clinical condition of ventricular dysfunction that develops in patients of type 2 diabetes mellitus. Obesity and insulin resistance are the main risk factors. The heart under this metabolically challenged condition manifests accumulation of free fatty acids, such as palmitate, generates lipotoxic stress, which decreases insulin sensitivity and adversely affects the activity of proteins responsible for glucose transport, including insulin receptor and Akt. Angiotensin-(1-9) is a peptide of the counter-regulatory axis of the renin-angiotensin system with cardioprotective effects, which reduces myocardial infarct size, blood pressure, and inflammation. However, it remains unknown whether angiotensin-(1-9) modulates insulin sensitivity in the heart on high-fat diet (HFD)-induced obesity.

Objective: To determine the role of angiotensin-(1-9) in insulin signaling in HFD-fed mice.

Methods: HFD-fed mice were treated with angiotensin-(1-9) for four weeks by osmotic mini pumps. Oral glucose tolerance tests were performed, and cardiac insulin sensitivity was assessed under the insulin injection. For statistical analysis, we used two-way ANOVA to compare four groups (Chow and HFD with or without angiotensin-(1-9)). Data were presented as mean + S.E.M. N=6 mice per group. All animal procedures were approved by the institutional ethics review committee of Universidad de Chile (code: 21444-CQF-UCH) and the IACUC of City of Hope (animal protocol: #21070).

Results: Angiotensin-(1-9) improved whole-body glucose tolerance after three weeks of treatment and recovered 50% of insulin receptor and Akt phosphorylation in the heart, but not the liver.

Conclusion: Angiotensin-(1-9) reverses the HFD-induced decrease in insulin sensitivity in the heart.

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P-16

Mitochondrial antioxidant mitoquinone's protective role in preventing hemodynamic alterations post-myocardial infarction in rats

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Introduction: cardiac mitochondria play a crucial role in cell survival and death following Myocardial Infarction (MI).

Objective: This study evaluated the effectiveness of the mitochondrial antioxidant mitoquinone (MitoQ) in preventing hemodynamic alterations 7 days after MI-surgery.

Methods: Animals were divided in Sham: n=11, MI: n=8, Sham MitoQ: n=6, and MI MitoQ: n=7 (Ethical Committee: CEUA-UFES 16/2021). During one week following MI, MitoQ groups received MitoQ diluted in drinking water (100 µM). On the 7th day arterial and ventricular pressure were measured through a catheterism. The catheter was inserted into the right common carotid artery measuring heart rate, arterial systolic blood pressure, and arterial diastolic blood pressure in mmHg. The catheter was advanced into the left ventricle (LV) to measure LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and the first derivatives (+/-dP/dt). Data are presented as mean ± SEM. Variables were compared by 2-way ANOVA and Tukey *post hoc*. Student's T-*test* compared the scar size. P values <0.05 were considered significant.

Results: MitoQ treatment prevented changes in diastolic blood pressure in mmHg (Sham: 84±3; MI: 68±3*; Sham MitoQ: 81±3; MI MitoQ: 85±3#) and LVEDP (Sham: 5±0.4, MI:10±1*; Sham MitoQ: 3±1; IM MitoQ: 5±0.5#), also LVSP and, dP/dt-. Analysis through Scanning Electron Microscopy showed the denudation of the aortic endothelium in MI, however treated animal endothelial denudation was not observed.

Conclusion: MitoQ presents a potential therapeutic efficacy as mitochondrial antioxidant in mitigating hemodynamic alterations and preserving endothelial integrity after MI.

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P-17

Mitochondrial MUL1 in experimental heart failure with preserved ejection fraction (HEpEF)

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Introduction: MUL1, a protein of the outer mitochondrial membrane with a zinc finger catalytic domain, regulates mitochondrial dynamics by controlling the levels and activities of Mfn2 and DRP1. Under physiological conditions, MUL1 sumoylates DRP1 and ubiquitinates MFN2, promoting mitochondrial fission. MUL1 is significantly expressed in cardiac tissue, favoring mitochondrial fragmentation and mitophagy, both cellular processes implicated in cardiovascular diseases. However, the effects of cardiomyocyte-specific MUL1 overexpression in HFpEF remain unknown, making it an attractive therapeutic target for potential therapies.

Objective: To evaluate the effect of cardiomyocyte-specific MUL1 overexpression on myocardial function in mice with HFpEF.

Methods: C57BL/6N and C57BL/6N-Tg(aMHC-Mul1) mice were assigned into groups: control diet and HFD + L-NAME (1.5 g/L) + NaCl (2%) (HFpEF) for 20 weeks (Bioethical protocol CICUA-CQyF2022-47). Cardiovascular and functional evaluations were conducted at the end of the treatment period. Mean ± SD, n=3-7 animals per group. Statistical analysis: ANOVA. A value of p>0.05 was considered significant.

Results: C57BL/6N-Tg(aMHC-Mul1) mice showed significant increases in cardiac MUL1. Diastolic fun ction (E/e ratio), hypertrophy (heart weight/body weight), blood pressure, and exercise tolerance tests were assessed, revealing significant differences between the groups. Through *in silico* analysis, a peptide was synthesized that could inhibit the catalytic action of MUL1, thereby inhibiting mitochondrial fission in vitro, with potential future therapeutic implications.

Conclusion: Cardiomyocyte-specific MUL1 overexpression could affect the parameters of myocardial fun ction. Further experiments in vitro and in vivo should contribute to elucidate the effect of the peptide on cardiomyocyte hypertrophy and myocardial fun ction

Financing: FONDECYT 1200490 (SL), FONDAP 15130011 (SL) and Ph.D. fellowship 21191903 from ANID, Chile.

P-18

Efficacy of Poloxamer 188 in reducing electric injury in human cardiomyocytes exposed to pulsed electric fields

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Introduction: Ventricular fibrillation, a critical cardiac condition, is treated primarily with electrical defibrillation. However, this method often damages cardiac cells through electroporation, contributing to high mortality in post-cardiac arrest syndrome. Poloxamer 188 (P188) has shown potential in preserving electroporated cell membranes, but its effectiveness during cardiopulmonary resuscitation (CPR) remains unclear.

Objective: We investigated the time course of cell death, the mechanisms involved, and the protective effects of P188 on AC16 human cardiomyocyte cell line exposed to microsecond and nanosecond pulsed electric fields (µsPEF and nsPEF).

Methods: Using a custom 3D printer with electrode holders, we positioned electrodes relative to cell monolayers in a nanofiber multiwell plate. nsPEF and µsPEF shocks produced a non-uniform electric field, allowing efficient measurements across electric field strengths. Cell viability and caspase 3/7 activation were assessed via fluorescence microscopy 2–24 hours post-treatment.

Results: nsPEF shocks resulted in minimal caspase 3/7 activation, with most lethally injured cells showing propidium dye permeability as early as 2 hours post-exposure. On the other hand, µsPEF shocks led to significant caspase 3/7 activation at the 2-hour mark, and the number of dead cells continued to rise up to 24 hours, indicating a predominance of apoptotic cell death. Application of P188 in concentrations ranging from 0.2–1% was shown to reduce cell death, highlighting its potential utility for in vivo applications to mitigate electric injury caused by defibrillation.

Conclusion: P188 shows promise in reducing electric injury in cardiac cells exposed to defibrillation shocks, potentially improving post-defibrillation outcomes.

Financing: We would like to acknowledge the Kosciuszko Foundation for granting Aleksander Kielbik the Research Award for an internship in the Frank Reidy Research Center for Bioelectrics.

P-19

Reduction in blood pressure, cardiac contractility and vascular contractile response in rat aorta by novel compound synthetized from Senecio nutans SCh. Bip. (Chachacoma)

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Introduction: *Senecio nutans* Sch. Bip (Chachacoma) is an endemic plant commonly employed in Andes culture to counteract the effects of hypertension, and its bioactive molecules could provide new drugs for treating hypertension.

Objective: To compare the cardiovascular and vascular response of a novel oxime synthesized from a metabolite of *Senecio nutans*.

Methods: We isolated a metabolite from *S. nutans* (4-hydroxy-3-(isopenten-2-yl)-acetophenone) (SG4) and synthesized its oxime-SG4. We evaluated the effects of oxime-SG4 on vascular reactivity, blood pressure in anesthetized preparation, and Langendorff in rats. Rats were

anesthetized (isoflurane 2%), and during continued recording, oxime-SG4 was administrated (i.v.) in the presence and absence of phenylephrine (PE). In other animal series, the hearts and aortic rings were extracted and isolated to determine contractility heart function, and vascular reactivity. Student test or ANOVA followed by *post-hoc* Bonferroni were used (p<0.05) for statistical analysis. Study was approved by the Ethics Committee of Universidad de Antofagasta (CEIC-275/20).

Results: Preincubation of aortic rings with oxime-SG4 (10^{-5} M) significantly (p<0.001) decreased the contractile response to 10^{-6} M of PE, whereas SG4 did not. This finding matches under a similar protocol in anesthetized rats, where oxime-SG4 (10^{-4} M) significantly reduced (p<0.05) the PE (25 µg/g)-dependent increase of mean arterial pressure (MAP). Both compounds, SG4 and oxime-SG4 reduced MAP and decreased left ventricular pressure (Langendorff) in normotensive rats.

Conclusion: A novel compound synthesized from *S. nutans* reduces MAP, cardiac contractility, and vascular contractile response in rat aortic rings.

Financing: Financial support was provided by FONDECYT 1200610 to J.P., the Network for Extreme Environments Research project to F.C. and A.P. (NEXER, Project ANT1756, Universidad de Antofagasta, Chile); Fondecyt de Iniciación 11220870 to DCA.

P-20

The lack of estrogens does not affect the heart in female mice with recently established menopause and insulin resistance

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Introduction: Previous reports described that male showed insulin-resistance (IR) and cardiac alterations after a fructose-rich diet (FRD). However, estrogen protection over the heart in reproductive females is controversial.

Objective: Identify the beneficial effects of estrogens in the IR context on the heart.

Methods: Groups: 1-2) Female rats or 3-4) mice with control diet (CD) or fructose-rich diet (FRD) (10% fructose) for 3-4 weeks. 5-6) Female mice underwent an ovariectomy (OVX) or placebo (SHAM) plus FRD. We performed metabolic, echocardiography, morphological and intracellular Ca2+ measurements. Data were compared by t-test in between groups, and p<0.05 was considered a significant difference. Bioethics Committee approval CICUAL P03-02-2017

Results: FRD did not induce IR or heart abnormalities in females at reproductive age. Groups 1-4 did not present differences in IR indexes, morphometric, or echocardiographic parameters. Estrogens are involved in IR but not in cardiac alterations (groups 5-6). OVX-FRD mice showed a significant difference (p<0.05) in metabolic parameters vs. SHAM-FRD, indicating an IR condition. However, morphometric and echocardiographic parameters did not reveal any difference between groups at the level of the heart. Estrogens are not involved in Ca2+ response after a challenge. OVX-FRD showed the same Ca2+ handling response (Ca2+ transient amplitude and dynamics) to 100nM Isoproterenol or an increase in stimulation frequency vs. SHAM-FRD.

Conclusion: FRD did not induce IR in the reproductive age of females, but the lack of estrogens would induce IR without heart abnormalities. We suggest that female hearts, under IR conditions, are protected by other molecules not derived from the ovaries.

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P-21

Exploring the effects of short and medium-term high-altitude exposure on cardiorespiratory and metabolic responses to incremental exercise testing

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Introduction: High-altitude regions worldwide, such as the Andes and Himalayas, attract athletes, tourists, and workers ascending above 3,000 meters for physically demanding activities. However, the influence of the autonomic nervous system on cardiovascular and respiratory responses to natural hypobaric hypoxia remains unclear.

Objective: This study aimed to evaluate the acute effects of high altitude on baroreflex sensitivity, cardiorespiratory, and metabolic responses to exercise.

Methods: Nine healthy volunteers completed maximal cardiopulmonary exercise tests (CPETs) at sea level (SL) and high altitude (3,025 m) at 12 (HA_{12h}) and 60 (HA_{60h}) hours after arrival. Continuous beat-to-beat hemodynamics, respiration, and gas metabolism were monitored. Baroreflex sensitivity was evaluated through the sequence method by analyzing spontaneous blood pressure and heart rate fluctuations. Statistical analyses included Friedman and Tukey's tests, as well as Spearman correlation (α=0.05). Ethics approval was granted by the Pontificia Universidad Católica de Chile (#220906004).

Results: During CPET, maximal exercise power, relative oxygen uptake, and carbon dioxide production volume were significantly lower at HA_{12h} and HA_{60h} than at SL, while the ventilatory equivalent for carbon dioxide (VE/VCO_2) increased. The oxygen pulse, end-tidal carbon dioxide, and VE/VCO2 exhibited significant differences between conditions at the anaerobic threshold and respiratory compensation point. Remarkably, baroreflex sensitivity inversely correlated with maximal systolic blood pressure.

Conclusion: These findings underscore the significant impact of short and medium-term exposure to high altitude on cardiorespiratory and metabolic responses to strenuous exercise. Additionally, the correlation between baroreflex sensitivity and the maximal vascular response to exercise suggests a promising avenue for further in-depth exploration.

Financing: This research was supported by ANID–ACT210083 and Fondecyt grants 1230844 and 11220870.

P-22 ★ *undergrad sci competition*

Mitochondrial antioxidant mitoquinone prevents vascular dysfunction, oxidative stress, and mitochondrial-NADPH oxidase crosstalk following myocardial infarction

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Introduction: Myocardial infarction (MI) is a condition triggering inflammatory responses and oxidative stress, leading to vascular and endothelial dysfunction.

Objective: Our research explores the role of mitochondrial-NADPH oxidase (NOX) crosstalk in aortic vascular reactivity 7 days post-MI.

Methods: Male Wistar rats underwent surgical MI and were categorized into Myocardial Infarction (MI), Myocardial Infarction Mitoquinone (MIM), Sham (S), and Sham Mitoquinone (SM). Animals received Mitoquinone (100 µM), a specific mitochondrial antioxidant, in drinking water for seven days (Ethical Committee CEUA-UFES 17/2021). Vascular reactivity was evaluated in isolated aortic rings under phenylephrine stimulation. Results are presented as mean±SEM, significant to $p<0.05$, ANOVA one-way plus Tukey.

Results: There was no change in infarct area with Mitoquinone treatment (MI=46.6±2.8; MI MitoQ=43.6±2.6%), but a prevention of weight loss in the MI group (MI=-6±2.7*; MI MitoQ=16±4 g*, *p<0.01). Reactivity to phenylephrine increased in the MI (Rmax S:100.1±5.5 vs MI:127.3±8.7*% KCl 75 mM, *p<0.05). Mitoquinone prevented the increase in vascular reactivity in the MI (Rmax MI:127.3±8.7 vs MIM:100±8.5*% KCl 75 mM, p<0.05). L-NAME 100 µM had no effect on Rmax in the MI, suggesting a decrease in NO bioavailability. However, when the MI group was treated with MitoQ, superfusion with L-NAME increased the response to phenylephrine, suggesting higher NO bioavailability. Mitoquinone treatment reduced mitochondrial and cytosolic ROS and increased vascular NO production. The specific NOX inhibitor, ML-171 5 µM, reduced vascular reactivity and decreased NOX1 expression in the MI group.

Conclusion: The mitochondrial antioxidant mitoquinone prevented vascular dysfunction, and mitochondrial oxidative stress 7 days after MI.

Financing: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (Fapes), CNPq, UFES

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P-23 ★*selected for oral communication*

Role of Angiotensin Converting Enzyme 2 in the cardioprotective effect of endothelial small extracellular vesicles

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Introduction: Endothelial small extracellular vesicles (sEVs) are cardioprotective against ischemia/reperfusion injury. However, the mechanism mediating this effect and whether these vesicles also exert an anti-inflammatory effect, remains to be elucidated. Angiotensin Converting Enzyme 2 (ACE2) is a protein with known cardioprotective functions, but its role in the protective effects of endothelial sEVs is unkown.

Objective: To evaluate the anti-inflammatory effect of endothelial sEVs and the role of ACE2 in their cardioprotective effect.

Methods: sEVs were isolated from HUVECs using size exclusion chromatography. Size and concentration of sEVs was obtained by NanoSight. Expression of CD81 and ACE2 was determined by ELISA, whereas morphology by Electronic Microscopy. The effect of sEVs was tested with a wound healing assay using A7r5 cells treated with TNF-a (10 ng/mL). Cardioprotection of sEVs was evaluated by determination of infarct size using adult C57BL/6 mice hearts in *ex vivo* ischemia/reperfusion (CICUA-CQyF2023-53) with or without 1 µM of MLN-4760 (ACE2 inhibitor). Data were presented as mean ± SD and analyzed using Kruskal–Wallis's test with Dunn's multiple comparison analysis. Significant differences were considered at $p<0.05$.

Results: sEVs presented a cup-shaped form and the expression of CD81 and ACE2 was 1.23±049 and 3.19±1.55 ng/ml, respectively. sEVs reduced migration of A7r5 cells triggered by TNF-a (48.74%±10.4 vs 34.79%±16.7 **$p<0.01$, n=4) and reduced infarct size (45.65%±7.8 vs 25.5%±2.6 n=4, **$p<0.01$), but this effect was lost upon co-administration with MLN-4760 (54%±6.2, ***$p<0.001$).

Conclusion: Endothelial sEVs can attenuate migration of A7r5 and reduce infarct size by a mechanism that partly depends on ACE2.

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P-24

Effect of MgCl2 supplementation on vascular reactivity in spontaneously hypertensive animals

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Introduction: Magnesium supplementation has been used in hypertensive patients.

Objective: We analyzed the effect of Magnesium supplementation on the vascular reactivity of hypertensive rats.

Methods: Spontaneously hypertensive rats (SHR) were divided into a $MgCl_2$-treated group (50 mg/kg/day $MgCl_2$ in drinking water for two months; SHR-Mg) and a control group (SHR). Vascular reactivity of aortic rings was evaluated in response to increasing concentrations of phenylephrine in the presence of ML171 (NADPH oxidase inhibitor, NOX), L-NAME (NOS inhibitor), with or without endothelium (E-), and CID (specific PKD1 inhibitor). The results were analyzed using one-way ANOVA or Student's t-test (Animal Ethics Committee at UFES:16-2020).

Results: The maximum response (Rmax) to phenylephrine was reduced in the SHR-Mg^2 group (SHR: 123.6±3.5, n=7; SHR-Mg: 97.5±2.7*% KCl 75 mM, n=7, *$p<0.05$). Endothelium denudation (E-) SHR-Mg group showed a lower contractility response compared to SHR E- group (SHR-Mg E-: 163.3±4.9*, n=10; SHR E-: 233.2±12.1, n=7, *$p<0.05$). After inhibition of NADPH oxidase and PKD1, the reactivity of SHR and SHR-Mg groups became equal, suggesting the involvement of oxidative stress and the PKD1 pathway in the SHR

group that was prevented by $MgCl_2$ supplementation.

Conclusion: The findings indicate that a 2-month supplementation with $MgCl_2$ reduces vasoconstrictor response in aortic rings of SHR dependent on NOX and PKD1 pathway. This suggests a potential clinical importance, as magnesium supplementation may impact vascular reactivity. The involvement of oxidative stress and the PKD1 pathway in mediating these effects further highlights their significance.

Financing: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (Fapes), UFES, CNPq

P-25

Angiotensin-(1-9) and Angiotensin-(1-7) improve cardioprotective effects of Del Nido cardioplegia

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Introduction: Del Nido cardioplegia (DNC), a crystalloid solution containing a high concentration of potassium is used as a single-dose antegrade infusion to induce cardiac arrest in cardiac surgery. DNC arrests the heart in the repolarization phase, decreasing cardiac metabolism and cell damage. However, DNC continues to face the challenge of offering greater protection, especially in patients with underlying heart diseases. Angiotensin-(1-9) and Angiotensin-(1-7), peptides of the counter-regulatory renin-angiotensin system, have shown cardioprotective effects in pre-clinical models of cardiac ischemia/reperfusion (I/R) which makes them potential candidates to improve the cardioprotective effect of cardioplegia.

Objective: to evaluate the cardioprotective effects of angiotensin-(1-9) and angiotensin-(1-7), as adjuvant of the DNC.

Methods: we used isolated adult rat hearts and neonatal rat cardiomyocytes subjected to I/R. Bioethics committee approval 0041 from Universidad Libre, Barranquilla-Colombia. Data were presented as mean ± S.E.M and analyzed by nonparametric test. Significant differences were considered $p<0.05$**.**

Results: The addition of DNC with angiotensin-(1-9) and angiotensin-(1-7) decreased lactic dehydrogenase (LDH) release, (43.2%±1.0 and 44.38%±3.7, respectively), in cultured cardiomyocytes subjected to simulated I/R (sI/R), in comparison to cardiomyocytes subjected to sI/R and incubated with DNC (60%±1.2, $p<0.05$, n= 4 independent experiments). Hearts treated with angiotensin-(1-9) and subjected to DNC+I/R showed fewer arrhythmias during reperfusion and recovered left ventricular function in a shorter time to reach pressure stability (5 min±0.6 vs 41 min±8.7, N=3).

Conclusion: Angiotensin-(1-9) and angiotensin-(1-7) decreased cell death in cardiomyocytes subjected to sI/R. Reperfusion with angiotensin-(1-9) improved the recovery of the left ventricular function of hearts subjected to I/R.

Financing: FONDAP 15130011 (SL), MINCIENCIAS-Colombia 120884468190 (EM), Puente-ICBM-570334 (GS), Fondecyt 1220325 (GS).

P-26

Evolution of blood pressure based on a diet rich in magnesium chloride in Spontaneously Hypertensive Rats (SHR)

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Introduction: Epidemiological studies suggest that a diet rich in Mg^{2+} may have a beneficial role in the prevention and treatment of hypertension.

However, the results of such studies remain inconsistent.

Objective: This study aimed to investigate the hypothesis that a diet supplemented with $MgCl_2$ modifies blood pressure in hypertensive rats.

Methods: Spontaneously hypertensive rats (SHR) were divided into: one group received a supplementation daily dose of 50 mg/kg of $MgCl_2$ (drinking water for two months) (SHR-Mg), while the control group received no treatment (SHR). Blood pressure (BP) was measured weekly using the tail-cuff plethysmography method. The recorded arterial pulses were individually analyzed using one-way ANOVA test, post hoc Tukey (UFES Animal Ethics Committee 16-2020).

Results: Systolic arterial pressure (SAP) at the beginning and at the end of 8 weeks of treatment were comparable between the SHR-Mg² group (Initial SAP SHR:156.9 ± 5.7 vs SHR Mg:167 ± 5.8 mmHg, n=11; end SAP SHR: 164 ± 4.9 vs SHR Mg:175 ± 5 mmHg, n=9). Diastolic blood pressure was not significantly affected by Mg2+ treatment. Initial and final heart rates (HR) did not show any significant differences between the groups (Initial HR SHR:360 ± 5 vs SHR Mg:345 ± 8 bpm, n=11 and End HR SHR:336 ± 7 vs SHR Mg:351 ± 5 bpm).

Conclusion: In conclusion, the results of this study indicate that an eight-week supplementation with $MgCl_2$ at a dose of 50 mg/kg/day did not lead to any significant changes in blood pressure or heart rate in SHR animals.

Financing: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (Fapes); CNPq, UFES.

P-27

Cardiorespiratory alterations in Alzheimer's: targeting neural control of autonomic function to improve disease outcomes

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Introduction: While therapeutic approaches targeting neurodegenerative mechanism associated with cognitive impairment in Alzheimer's disease (AD) have been intensively explored, other comorbidities closely linked to AD progression like sleep-disordered breathing and autonomic dysfunction have not been thoroughly investigated.

Objective: To characterize cardiorespiratory function both in AD patients and in experimental model showing AD-like pathology (APP-PS1 transgenic mice).

Methods: 16 subjects (8 AD and 8 age-matched controls) were enrolled to assess sleep breathing disorders and to record cardiac autonomic function. Whole-body plethysmography and blood pressure monitoring in freely moving mice were used to study sleep-associated cardiorespiratory disorders. Experimental procedures were approved according to Animal Care and Use Committee protocol #490/2023/UACh.

Results: Compared to age-matched healthy subjects, patients with AD showed larger increases in neural sympathetic discharges during autonomic testing. In addition, heart rate variability analysis in AD patients showed a shift in spectral components towards a more sympathetic influence. Remarkably, we found that autonomic function impairment in the APP/PS1 mice resembles what we found in AD patients. Indeed, compared to wild-type mice (WT), APP/PS1 mice displayed a higher heart rate response (~55%) to systemic administration of a beta-blocker, sleep fragmentation and a high incidence of breathing disorders. Partial ablation of medullary sympathetic premotor neurons (~60%) in APP/PS1 mice results in two-fold reduction in cardiac sympathetic drive, marked improvements in breathing regularity and a restoration of normal sleep efficiency compared to untreated APP/PS1 mice.

Conclusion: Neural hyperactivity of sympathetic premotor region play a main role in the development/maintenance of cardiorespiratory disorders in AD.

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P-28

Cardiomyocyte vascular cell adhesion molecule 1 (VCAM-1) prevents cardiac dysfunction in obesity-induced cardiomyopathy

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Introduction: Obesity-induced cardiomyopathy (OIC) is a clinical condition with detrimental effects on cardiac function, but the molecular mechanisms are not thoroughly studied. Vascular Cell Adhesion Molecule-1 (VCAM-1) is a protein involved in the transmigration of inflammatory cells, but the role of VCAM-1 in OIC remains unknown.

Objective: To investigate the role of VCAM-1 in an experimental model of OIC in cardiomyocyte-VCAM-1-knockout animals (cVCAM-1-KO).

Methods: C57BL/6N cVCAM-1 KO and f/f male mice were fed with control or HFD for 25 weeks (Bioethical protocol CBE2020-04, U.Chile). We evaluated body, adipose tissue and heart weight, cardiomyocyte cross-sectional area (CSA), fibrosis, and echocardiography. We performed exercise and glucose tolerance tests, ELISA for soluble insulin and VCAM-1, RT-qPCR for ANP, BNP, and β-MHC mRNA levels, and Immuno-blot for VCAM-1 and pAKT(Ser473). Statistical analysis: mean±SD was used in n=3-15 and analyzed by non-parametric one-way ANOVA. $p<0.05$ was considered statistically significant.

Results: Mice fed with HFD developed obesity. The cVCAM-1-KO mice fed with HFD have diastolic and systolic dysfunction, exercise intolerance, and pathological cardiac hypertrophy, increasing heart weight, fibrosis, CSA, and mRNA expression of ANP and BNP, compared with the f/f HFD mice. Both HFD groups presented glucose intolerance. However, hyperinsulinemia was only increased in f/f HFD mice. Also, cVCAM-1-KO HFD mice show increased pAKT(Ser 473) under an insulin pulse. Soluble-VCAM-1 increased in f/f HFD animals but not in cVCAM-1-KO HFD.

Conclusion: c-VCAM-1-KO mice fed with HFD exacerbate the hypertrophic phenotype induced by HFD, suggesting that VCAM-1 has a protective role in a model of OIC.

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P-29

Assessment of vascular remodeling in a mouse model of heart failure with preserved ejection fraction

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Introduction: Heart Failure with preserved ejection fraction (HFpEF) is a syndrome characterized by a diastolic dysfunction with a high prevalence in Chile. HFpEF is associated with comorbidities such as obesity and hypertension. Macro and microvascular dysfunction are normally found in HFpEF patients. However, vascular remodeling is not described in mouse models of HFpEF.

Objective: To evaluate vascular remodeling in aortic tissue obtained from a murine model of HFpEF.

Methods: Aortas were obtained from a HFpEF mouse model feeded with a high fat diet (HFD, 60% Fat) and Nω-nitro-l-arginine methyl ester (L-NAME, 1,5 g/L). The experimental groups were: control (chow diet),obese (HFD), and HFpEF (HFD + L-NAME). In aorta tissue sections, morphology and fibrosis were assessed by hematoxylin-eosin and Picrosirius red staining, respectively. This work was approved of the bioethics committee of Faculty of Chemical and Pharmaceutical Sciences, University of Chile certificate Nº: 20412 – CYQ – UCH

Results: Aortic tissue of HFpEF mice presented a significant thickening of the arterial wall (from 67,11± 5,7 µm to 75,43± 7,9 µm $P<0.05$ ANOVA Kruskal-Wallis test $n=22$) and focal areas with vascular remodeling. However, significant changes in the media and lumen ratio in these conditions were not observed. Moreover, although collagen positive areas were observed in aortic tissue from HFpEF mice, these areas were not significantly different from HFD and control mice.

Conclusion: In aortic tissue from HFpEF mice there were increased levels of vascular remodeling in comparison to control mice.

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P-30

Evaluation of nucleotide variations in the placental ADORA2A gene and its association with preeclampsia

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Introduction: Preeclampsia (PE) is associated with reduced functional placental expression of the adenosine A2A receptor. Whether this reduction is linked with single nucleotide variations (SNVs) of the *ADORA2A* gene, including the SNVs involved in angiogenic processes is unknown.

Objective: To evaluate placental single nucleotide variations (SNVs) in the *ADORA2A* gene and their association with PE diagnosis.

Methods: The local Ethics Committee approved (registration number 596/022) this case (women with PE, n=50) and control (healthy pregnant women, n=50) study. PE was classified as early and late-onset. Placental DNA was used to evaluate five SNVs in *ADORA2A*: rs2298383, rs4822489, rs2236624, rs8192446, and rs17650937 by real-time PCR using TaqMan probes. The association between SNVs and PE was evaluated by multivariate regression analysis. Variables are expressed as mean ± SD.

Results: According to clinical criteria, we found high blood pressure in PE ($p<0.05$), particularly in late-onset PE compared with controls. Also, the frequency of obesity (BMI ≥30 kg/m^2) was significantly higher in late-onset than in early-onset PE ($p<0.05$). No significant differences were found in the genotypic and allelic frequencies of the studied SNVs in PE compared with controls. Also, SNVs and PE (late or early-onset) were not associated with any inheritance patterns analyzed.

Conclusion: No association between PE and the studied placental SNVs of the *ADORA2A* gene was found, nor was the analysis stratified in early-onset and late-onset PE. Future studies need to include a large population and sequencing analysis of the *ADORA2A* gene.

P-31

Impact of ω3 administration in the development of the slow force response in a menopause model

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Introduction: Myocardial stretch induces a rapid increase in force, followed by slow force response (SFR). The Na+/H+ exchanger (NHE1) activation and reactive oxygen species (ROS) production are crucial for its development. Omega-3 (ω3) fatty acids administration is cardioprotective. NHE1 activity and expression are enhanced in ovariectomized (OVX) rats.

Objective: Characterize the impact of ω3 administration on SFR development in OVX rats.
Methods: Two-month-old female rats were randomized into two groups: Sham (S) and OVX. After a month, some OVX received 200 mg/kg/day ω3 orally, for 3 months (OVXω3). Echocardiographic parameters and systolic blood pressure (SBP) were determined. After 3 months, SFR development in isolated papillary muscles, ROS production and NHE1 expression in left ventricular (LV) slices were evaluated. The data are presented as mean±SEM. Two-way ANOVA was performed and $p<0.05$ was considered significant. Experimental protocol was approved by local Committee (CICUAL #T05-01-2022)
Results: Ovariectomy produced uterine atrophy and body weight increase without affecting SBP. LV mass index was higher in OVX (mg/mm: 20.2±1.0, n=13 vs S), and ω3 significantly reduced it (17.7±0.6, n=15). SFR was similar in S and OVX (115.7±1.8%, n=8; 116.8±1.7%, n= 8, respectively), but was absent in OVXω3 (101.3±0.9%, n= 10). Basal ROS and NHE1 expression were greater in OVX (respectively, 187.7±21.4% n=9, 165.3 ± 17.1% n=8, vs S). Supplementation with ω3 significantly reduced them (134.3±12.8% n=10 and 124,6±7.8% vs OVX).
Conclusion: ω3 supplementation in OVX abolished SFR, probably as a consequence of a decrease in both ROS production and NHE1 expression.
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P-32

Cardiac dysfunction and oxidative stress after myocardial infarction are prevented by treatment with mitochondrial antioxidant mitoquinone

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Introduction: Myocardial infarction (MI) is a major cause of heart failure, and oxidative stress in the non-infarcted myocardium contributes to ventricular remodeling after MI. At 7 days post-MI, altered calcium handling and reactive oxygen species (ROS) are associated with decreased myocardial contractility. However, the specific role of mitochondrial ROS in contractile dysfunction during the early phase of MI is unknown.
Objective: This study investigated whether treatment with the mitochondrial antioxidant MitoQ, for 7 days after MI, would prevent contractility dysfunction.
Methods: Male Wistar rats were divided into: Sham, Sham MitoQ, MI, and MI MitoQ, administered for 7 days in the drinking water (100 µM). Myocardial contractility was measured in isolated papillary muscles, and ROS production was assessed. Statistical analysis was performed using two-way ANOVA and Tukey's post-hoc test (Ethical Committee 17/2020).
Results: MitoQ treatment did not affect the infarcted area size (MI=46.6 ± 2.8; MI MitoQ=43.6±2.6%) but prevented the decrease in weight gain (MI=-6±2.7*; MI MitoQ=16±4 g*, $p<0.01$). MitoQ preserved force, maximum positive derivative of force, and responsiveness to extracellular calcium in the MI group (Force in Ca^{2+} 1.25 mM: Sham= 0.56±0.06; MI= 0.29±0.5; MI MitoQ= 0.73±0.08* g/mg*, $p<0.05$). Additionally, MitoQ treatment reduced extracellular and mitochondrial ROS production, restoring redox homeostasis.
Conclusion: In conclusion, treatment with MitoQ for 7 days reduced mitochondrial ROS levels, preserved contractility function, and prevented the decline in functional parameters of isolated papillary muscles in the early phase of MI. These findings highlight the potential of mitochondrial antioxidant therapy in ameliorating contractility dysfunction following MI.
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AREA: ENDOCRINE AND METABOLISM

P-33

Decreased expression (mRNA) of markers of chronoregulation Rev-erbα and Clock in metabolically healthy vs. unhealthy obesity

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Introduction: Obesity is defined as a body mass index (BMI) ≥30kg/m^2, however based on the wide interindividual variability in metabolic health, metabolically healthy (MHO) and unhealthy (MUO) obesity phenotypes have been defined. Peripheral blood mononuclear cells (PBMC) reflect changes associated with cardiometabolic dysfunction through gene expression. Evidence on the association between obesity and molecular clock regulation, indicates that the expression of the factors *Rev-erbα* and *Clock* could modulate adipose metabolism and inflammation.

Objective: To determine whether mRNA expression of the molecular clock genes *Rev-erbα* and *Clock* in PBMC is different between MUO and MHO, and its correlation with lipid regulation and proinflammatory marker expression and anthropometric parameters.

Methods: Adults were classified as MHO (n=6) or MUO (n=22) according to BMI, waist circumference (WC) and blood pressure reference values. PBMC were isolated (StemCell Technologies protocol), and mRNA transcripts were evaluated by qPCR. Statistic analysis included Wilcoxon (Mann Whitney) and Spearman tests. Subjects signed the informed consent (INTA's Ethics Committee #15-2021).

Results: MHO showed a 1.5 and 2.4-fold higher mRNA expression of *Rev-erbα* and *Clock* respectively, vs MUO ($p<0.05$). There was a negative correlation between WC and both *Rev-erbα* (r=-0.4122) and *Clock* (r=-0.4102) ($p<0.05$) mRNA. *Rev-erbα* showed significant positive correlations with *TNFα*, *FASN* and *PPARα*, and *Clock* with *TLR4*, *IL1β*, *ACC1* and *PPARα*.

Conclusion: PBMC from subjects with MUO show lower gene expression of *Rev-erbα* and *Clock* compared to MHO. Subjects with larger WC present lower mRNA expression of *Clock* and *Rev-erbα.*

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P-34

Fibroblast growth factor 21 (FGF21) gene deletion worsens insulin resistance in murine models of severe obesity and generalized lipodystrophy

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Introduction: The fibroblast growth factor 21 (FGF21) is a hormone mainly expressed in the liver. Although FGF21 administration in obesity models improves glucose tolerance, insulin sensitivity and reduces hepatic triglyceride (TGs) levels, its physiological roles remain less understood. Furthermore, FGF21 is elevated in models of insulin resistance (IR), diabetes, and its levels positively correlate with BMI in humans.

Objective: To assess the physiological actions of FGF21 in the metabolic phenotype of murine models with severe IR associated with obesity (*db/db*) and lipodystrophy (*Agpat2*$^{-/-}$).

Methods: *db/db;Fgf21*$^{-/-}$, *Agpat2*$^{-/-}$*;Fgf21*$^{-/-}$ and control mice were fed *ad libitum* a standard chow. Data were expressed as mean ± SD. Statistical significance was determined by one-way ANOVA (n=3-7). Animal experiments were approved by the Pontificia Universidad Católica de Chile's IACUC (190930006).

Results: *db/db;Fgf21*$^{+/+}$ and *db/db;Fgf21*$^{-/-}$ mice were ~2-fold heavier than *WT;Fgf21*$^{+/+}$ and *WT;Fgf21*$^{-/-}$ mice ($p>0.001$). However, the normalized liver weight of *db/db;Fgf21*$^{-/-}$ mice was ~20% heavier than *db/db;Fgf21*$^{+/+}$ ($p>0.001$). *Fgf21* deletion did not change body weight, plasma glucose nor TGs levels in *Agpat2*$^{-/-}$ mice. Insulinemia in *Agpat2*$^{-/-}$*;Fgf21*$^{-/-}$ mice was ~4-fold larger than in *Agpat2*$^{-/-}$*;Fgf21*$^{+/+}$ mice and ~103-fold larger than *Agpat2*$^{+/+}$*;Fgf21*$^{+/+}$ and *Agpat2*$^{+/+}$*;Fgf21*$^{-/-}$ mice ($p>0.001$). *Agpat2*$^{-/-}$*;FGF21*$^{-/-}$ mice had larger livers and increased hepatic TGs and cholesterol levels than *Agpat2*$^{+/+}$*;Fgf21*$^{+/+}$ and *Agpat2*$^{+/+}$*;Fgf21*$^{-/-}$ mice ($p>0.001$) with no differences compared to

Agpat2$^{-/-}$;*Fgf21*$^{+/+}$. No alterations in the insulin signaling pathway proteins were observed. **Conclusions:** *Fgf21* deletion results in increased hepatomegaly in *db*/*db* and increased hyperinsulinemia in *Agpat2*$^{-/-}$ mice. These results suggest that FGF21 in murine models of hyperinsulinemia, diabetes, and fatty liver ameliorates IR.

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P-35

Differences of sex on endothelial cells in response to fructose

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Introduction: Fructose is a common sugar that has been linked to metabolic, and cardiovascular diseases. Some studies have suggested that excessive intake of fructose may have harmful effects on the kidneys and blood pressure, which are risk factors for cardiovascular and kidney disease. Additionally, it has been observed there are differences between sexes in the incidence and expression of these pathologies. However, the effects of fructose on endothelial function and sex differences are not well understood. **Objective:** to analyze the transcriptome of ECs to identify pathways that may determine the sex differences in the effect of fructose on the metabolism.

Methods: We investigated the pathways involved in fructose metabolism on EC obtained from the human umbilical vein from three male and female donors (Ethics and Biosafety Committee at PUC 150730038). We used RNA sequencing to analyze the transcriptomic profiles of human ECs using GSEA. We performed functional assays to measure the viability and migration of EC in response to treatment with 5 mmol/L of fructose or glucose for 48 hours (Student's t-test (n=3)).

Results: We identified differentially expressed genes and cell clusters related to fructose metabolism and sex origin. We found that fructose-induced sex-specific changes in the expression of genes involved in inflammation and angiogenesis. Also, we found that fructose reduced the viability and migration of ECs from both sexes, but more markedly in males. **Conclusion:** These results suggest that fructose induces sex-specific alterations in endothelial function, which may contribute to the development of vascular complications associated with fructose intake.

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P-36

Hypothalamic bioenergetics and metabolic control: Contribution of mitochondrial function, AMPK and KATP channels

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Introduction: Obesity is a global public health problem. The hypothalamic arcuate nucleus regulates body energy balance (BEB), whose activity is altered in obesity. This could be the product of an alteration in mitochondrial function, AMPK, and KATP channels, whose restoration of their function would regulate the BEB.

Objective: To evaluate whether the obesity-induced metabolic risk phenotype is prevented with metformin, by restoring mitochondrial function, AMPK activity, and K_{ATP} channels, in the hypothalamus of mice exposed to an obesogenic environment.

Methods: Four-week-old C57BL/6 mice were fed a standard diet (D10%), a high-fat diet (D45%), or a D45%+300 mg/kg/d metformin (D45%+MT) for 12 weeks. Body weight, food intake, respiratory exchange ratio, and locomotor activity were measured, and an intraperitoneal glucose tolerance test (IGTT) was performed. Expression of mitochondrial genes and proteins were measured by qPCR and Western Blot, respectively. ATP and ROS levels were measured by commercial kits. Data were expressed as mean ± SEM (n= 3-6, One

Way and repeated measures ANOVA). This work was approved by the Institutional Committee on Ethics in Animal Care and Use of the San Sebastián University, code 062022.
Results: Treatment with metformin decreased body weight gain, food intake, increased locomotor activity, and normalized glycemia in the IGTT, compared to the D45% group. Treatment with metformin normalized the levels of mitochondrial membrane potential and ROS in the cytoplasm and tended to increase mitochondrial ATP production.
Conclusion: These results suggest that obesity induces bioenergetic changes in the hypothalamus prevented by metformin by promoting greater control of the BEB.
Financing: Fondecyt 1181574, 1230905 (BK), Fondecyt 11200592 (MJY), Anillo ACT210039 (UC-USS), ANID 21190474 (AV).

P-37

Exacerbated inflammatory gene transcript response to LPS in individuals with metabolically unhealthy vs. healthy obesity

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Introduction: Obesity is a major public health concern, associated with metabolic disorders. However, a wide inter-individual variability in its cardiometabolic morbidity has led to defining "metabolically healthy obesity (MHO)" (obesity with adequate metabolic health), as opposed to the classical metabolically unhealthy obesity (MUO). Peripheral blood mononuclear cells (PBMC) are circulating immune cells exposed to systemic environment and a non-invasive proxy for metabolic effects. Toll-like receptor (TLR)4 activation is relevant in obesity-induced inflammation, and its response to lipopolysaccharide (LPS) reflects an individual's proinflammatory state.
Objective: To determine whether LPS exposure in PBMC induces a different proinflammatory gene expression response between MHO and MUO.
Methods: PBMC were isolated (LymphoprepTM) from adults classified as MHO or MUO, according to body mass index, waist circumference and blood pressure reference values. Cells were treated (10 ng/ml LPS or vehicle) for 3h, and cDNA was obtained to determine proinflammatory factor mRNA (qPCR). Data from 6-7 MHO and 14-15 MUO were analysed using Wilcoxon and Mann-Whitney non-parametric tests. Subjects signed the informed consent (INTA's Ethics Committee #15-2021).
Results: MUO showed 708%, 193% and 489% greater increase in TNF-α, IL-6 and IL1β mRNA expression, respectively, upon LPS exposure vs.MHO ($p<0.05$). There was a 9.2-, 13.3-, 4.6-, 4.4- and 12-fold increase in TNF-α, IL-6, IL-1β, NLRP3 and CASP1 mRNA, respectively, upon LPS treatment in MUO ($p<0.05$), with no significant response in MHO. There was no effect of LPS on CCL2 or TLR4 expression.
Conclusion: PBMCs from subjects with MUO show a greater inflammatory response to LPS than MHO.
Financing: This work was supported by FONDECYT 1211477 to MC. and FONDAP 15130011 to MC. from Agencia Nacional de Investigación y Desarrollo (ANID), and Beca Doctorado Nacional ANID-Chile (N° 21220077) to GY.

P-38

Effects of early-postnatal overfeeding and peripubertal metformin administration on the puberty onset and estrous cycle of female Wistar rats

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Introduction: Lactation and adolescence are developmental plastic windows where environmental stressors may trigger long-term consequences for metabolism and reproduction functions, as Developmental Origins of Health and Disease (DOHaD) studies show.
Objectives: We aimed to investigate the effects of early postnatal-overfeeding induced by litter

reduction and metformin administration during peripuberty on the puberty onset and the estrous cycle of Wistar rats.

Methods: Ethical Approval CEUA No 4831020822. Delivery was postnatal-day 0 (PN0), at PN3 Wistar rats' litters were adjusted for 9 (NL – N=8) or 3 (SL – N=8) pups per dam. At PN21 litters were weaned. At PN30 metformin treatment was introduced via drink water (300mg/kg of body weight) until PN60, setting 4 groups (N=4 each): NL-WAT, SL-WAT, NL-MET and SL-MET. Following vaginal opening, the estrous cycle was monitored for 30 days by observation of the vaginal smear. Statistical analysis performed by Two-way ANOVA with data presented as Mean ± S.E.M.

Results: Early postnatal-overfeeding induced obesity in peripubertal Wistar female rats (PN30, NL 79,75±11,85 vs SL 98,52±9,95, $p<0.001$) and decreased the number of regular estrous cycles (NL-WAT 50±4.7% vs SL-WAT 25±5.2% $p<0.0001$). Metformin reduced body-weight in SL rats (PN60, SL-WAT 226,5±5,08 vs SL-MET 217,3±15,38, $p<0.0001$) delayed puberty onset for NL and SL (Metformin effect $p<0.001$), and, increased regulated estrous cycles in SL puberty (SL-WAT 25±5.2% vs SL-MET 33.8±3.7%, $p<0.0001$).

Conclusion: Early postnatal-overfeeding negatively affected the regulation of estrous cycle in pubertal female rats, while metformin intervention delayed puberty onset, decreased body-weight and regulated estrous cycle in SL rats.

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P-39

Getting a model of diabetes mellitus in guinea pigs for the study of diabetic cardiomyopathy

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Introduction: Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from defects in the insulin level. The main types are 1 and 2, the latter being more frequent. One of the complications with the worst prognosis, which can lead to death, is diabetic cardiomyopathy (CMD).

Objective: We decided to investigate the guinea pig model due to mice limitations. The protocol was approved by the Honorary Commission for Animal Experimentation (number 070153.00084619).

Methods: Two groups of equal age and sex were maintained and compared for three months, one being a control and the other treated with streptozotocin (STZ), alloxan (ALX), a hyperlipidic and hyperglycemic diet. Temporal variation in weight, fluid intake, glycemia, and electrocardiogram (ECG) was compared between groups for two to three months.

Results and conclusion: unpaired-samples-T-test mean+/-SEM 6. Weight and fluid intake increased 20% to 30% from the first month in treated patients and glycemia 2 to 3 times inconsistently over time from the second month. 66% of those treated showed ECG changes of deviation from the median axis type hypertrophy. The latter were sacrificed, and cardiac function results of isolated hearts were compared at baseline and in ischemia reperfusion, with less functional reserve and being more prone to arrhythmias. It was possible to obtain diabetic type II guinea pigs in approximately three months. In addition, it is possible to characterize in vivo if they present CMD, so the isolated hearts come from animals that we suspect have DM and CMD

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P-40 ★ *undergrad sci competition*

Long-term lithogenic diet induces steatohepatitis and increases circulating bile acids in female mice

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Introduction: Lithogenic diet (LD) has been helpful in preclinical studies to induce gallstone disease (GSD), mostly in males but not females. GSD is a prevalent disease and affects more women than men. GSD affects bile acids (BA) metabolism; it could induce hepatic damage or steatohepatitis. Steatohepatitis and BA levels behind long-term exposure to LD are poorly understood.
Objective: Evaluate whether circulating BA correlates with steatohepatitis in female mice long-term fed LD.
Methods: All experiments were conducted according to the Bioethics Committee of Universidad de Chile (CICUA-CQyF2021-34). Eight-week-old female C57/BL6N mice were assigned into two groups): Chow diet (Control; n=3-5) and LD (1.25% cholesterol, 0.5% cholic acid); (GSD; n=3-5) to promote GSD. After nine months, liver, spleen and plasma were collected. Hepatic tissue and gallbladder were collected for analysis. Statistical analysis was performed with the Mann-Whitney test.
Results: GSD increased liver weight (32±2 vs 76±12 mg/g, p=0.004), spleen weight (2,5±0,2 vs 5,1±1,6, p=0.004), hepatic triglycerides (1687±371 vs 2909±216 ng/nmol, p=0,010) compared to controls. Liver hematoxylin-eosin staining showed diffuse inflammatory infiltrate and steatosis in GSD. NAFLD activity score (NAS) that evaluates ballooning, steatosis, inflammation and fibrosis, also increased in GSD (0,2±0,4 vs 11±1, p=0,008). Total BA were increased in GSD (682±373 vs 6518±1519 ng/mL, p=0.003).
Conclusion: Long-term LD promoted liver inflammation, fibrosis, steatosis and ballooning. Future research should determine the molecular mechanism associated to GSD and its relationship with steatohepatitis and BA metabolism in female mice.

P-41

Women with gestational diabetes show unstable glycaemia dynamics late in pregnancy

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Introduction: Continuous glucose monitoring (CGM) allows for studying the kinetics of changes in glycaemia, i.e. glycaemia dynamics. A few numbers of CGM metrics are reported in pregnant women with normal or gestational diabetes mellitus (GDM) pregnancies.
Objective: To determine the glycaemia dynamics in pregnant women with GDM.
Methods: CGM FreeStyle Libre TM device was used for glycaemia recording (up to 14 days) in a woman with normal (34 weeks of gestation, wg) and GDM (32 and 34 wg) pregnancy (Clinical Hospital UC CHRISTUS, with patient consent, Ethics #012793). The CGM metrics determined were average (*X*), standard deviation (SD), coefficient of variation (%CV), interquartile range (IQR), percentage of time in range (%TIR), distance travelled (DT), low (LBGI) and high (HBGI) blood glucose index.
Results: Women with GDM showed higher *X* (172 vs 91 mg/dL), SD (34 vs 25 mg/dL), %CV (27 vs 20%), IQR (40 vs 30 mg/dL), DT (4630 vs 3008 mg/dL), and HBGI (7.480 vs 0.173) compared with normal pregnancies. GDM is also associated with lower %TIR (65 vs 85%) and LBGI (0.001 vs 4.593).
Conclusion: Pregnant women with GDM show unstable control of their glycaemia late in pregnancy which could explain a higher time out of the desired glycaemia range.
Financing: VRI DIDEMUC-PUC (Chile) and Sao Paulo Research Foundation-FAPESP [Grant-FAPESP 2016⁄01743–5] (Brazil). MC and GF hold fellowships from the ATTP-UMCG, and De Cock-Hadders (MC n°2023-51, GF n°2022-12/2023-29). GF, MC, and PV hold PhD fellowships from ANID [21221950, 21222280, and 21221870] and U Talca (Chile).

P-42 ★ *undergrad sci competition*

Hydrogen sulfide reverses gestational diabetes mellitus-altered expression of human equilibrative nucleoside transporters in fetoplacental endothelium

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Introduction: Hydrogen sulfide (H_2S) and extracellular adenosine regulate nitric oxide (NO) synthesis in human umbilical vein endothelial cells (HUVECs).

Objective: To determine whether H_2S modulates human equilibrative nucleoside transporter 1 (hENT-1) and hENT-2 expression in HUVECs from gestational diabetes mellitus (GDM).

Methods: HUVECs were from women with normal pregnancies and pre-pregnancy normal weight (N*nw*) or obese (N*ob*) (n = 3-5) and GDM*nw* or GDM*ob* (n = 3) (Clinical Hospital UC CHRISTUS, with patient consent, Ethics #012793). Cells were exposed (13 h, 1-30 mmol/L) to NaSH (H_2S donor) or PAG (H_2S inhibitor). hENT-1 and hENT-2 protein abundance was determined by Western blot. Data was analyzed by two-way ANOVA, mean ± SEM, with $P<0.05$ as significant.

Results: HUVECs were from women with normal pregnancies and pre-pregnancy normal weight (N*nw*) or obese (N*ob*) (n = 3-5) and GDM*nw* or GDM*ob* (n = 3) (Clinical Hospital UC CHRISTUS, with patient consent, Ethics #012793). Cells were exposed (13 h, 1-30 mmol/L) to NaSH (H_2S donor) or PAG (H_2S inhibitor). hENT-1 and hENT-2 protein abundance was determined by Western blot. Data was analyzed by two-way ANOVA, mean ± SEM, with $P<0.05$ as significant.

Conclusion: Exogenous H_2S may be protective to GDM-altered hENT1/hENT2 protein abundance in HUVECs.

Financing: VRI+DIDEMUC PUC, ANID 21221870, U Talca Chile.

AREA: IMMUNOLOGY AND CANCER

P-43 ★*selected for oral communication*

Protective effect of alamandine in a melanoma solid tumor and cancer associated cachexia model

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Introduction: The Renin Angiotensin System (RAS) in relevant in cancer and cachexia research. RAS peptides have been related to anti-tumoral and anticachectic effects.

Objective: Investigate the antitumoral and anticachectic effect of Alamandine (Ala).

Methods: C57BL/6 mice were injected with B16F10 (Animal Ethics Committee approval: 198/2018) and divided into Control (n=5) and the treatment (Ala 50µg/Kg/day; n=5). Mice weight and tumor volume were monitored, plasma and melanoma tissue samples were collected to measure the levels of circulating peptides. Muscle and adipose tissue were collected to analyze the cachexia index. Statistical analysis performed using One Way (post hoc Tukey) or Two-way ANOVA (post hoc Bonferroni). Data represented as ± S.E.M.

Results: We found a significant reduction in tumor mass/volume by Ala treatment. Decreased abundance of ERK1/2 and increased abundance of ACE2 in melanoma tissue of Ala treated mice. Moreover, the Ala group exhibited lower levels of AngII in the melanoma tissue, along with higher levels of circulating Alamandine and AngI. The body mass of the animals in the treatment group was preserved compared to their initial body mass. The percentage of muscle and fat mass relative to the initial body mass demonstrated the following results: Gastrocnemius muscle: CT 4.03±0.12% vs. Ala 4.82±0.21%; Tibialis anterior muscle: CT 1.16±0.06% vs. Ala 1.65±0.13%; Soleus muscle: CT 0.20±0.01% vs. Ala 0.32±0.025%; Heart muscle: CT 3.67±0.08% vs. Ala 4.55±0.18%; Epididymal fat pad: CT 0.61±0.27% vs. Ala 4.29±1.14%.

Conclusion: Our data reveals an anti-tumoral and anticachectic effect of an oral formulation containing Alamandine in tumor bearing mice.

Financing: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), INCT NanoBiofar.

P-44

Antitumoral effects of a botanical extract of Agaraphytum chilensis in prostate cancer

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Introduction: Prostate cancer (PCa) represents the second leading cause of cancer-related deaths in men in the majority of western countries. The current therapies to counteract PCa are very limited, highly ineffective, and generate significant side effects. The search for new molecules with therapeutic potential has drawn the attention towards the study of the polyphyletic group of algae, which constitutes an interesting source of raw material for the search of compounds against cancer.

Objective: Studied the effect of a new botanical extract of *Agarophytum chilensis* (Gracilex®) as a potential therapeutic agent against PCa cells.

Methods: The *in vitro* effect of Gracilex® were performed with LNCaP and PC-3 cell lines (*n*=3). The *in vivo* effect of Gracilex® was analyzed using a xenograft model of PCa (*n*=3) (approved by animal bioethics-PUC n°210126003). For statistical analyses, we used Prism v.9. program (unpaired t-test). p-value <0.05 were considered statistically significant.

Results: LNCaP and PC-3 cell lines, indicated that this extract inhibited, in a dose-dependent manner, cell survival in both cell types. In addition, Gracilex® increased apoptosis of PC-3 cells. Growth curves of the PC-3 cell line evidenced a significant decrease in the exponential growth phase slope and the stationary phase, which suggests both, a direct inhibitory effect of Gracilex® on the proliferation rate and the recovery of the contact inhibition capacity, respectively.

Conclusion: Our results indicated that Gracilex® significantly decreased tumor growth by inhibiting tumor cell proliferation. Together, our data support an antitumoral effect of Gracilex® on PCa cells.

Financing: CORFO-IFAN 16PTECAI–66648.

P-45

Vitamin D promotes glutaminolysis in CD4+ T lymphocytes

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Introduction: Vitamin D is an essential immunomodulating fat-soluble hormone that regulates functional responses in several cells, including $CD4^+$T-cells. Activated $CD4^+$T-cells preferentially uses glycolysis as its main metabolic pathway to proliferate and exert pro-inflammatory responses. The Vitamin D receptor is also upregulated after T-cell activation and it has been shown that Vitamin D increases $CD4^+$ T-cell proliferation and survival, but reduces glucose uptake, suggesting that an alternative metabolic pathway is required to maintain T-cell survival.

Objective: The aim of this project is to identify which metabolic pathway is promoted by Vitamin D to support $CD4^+$T-cell metabolism to compensate the reduced glycolysis.

Methods: $CD4^+$T-cells were isolated from peripheral blood and cultured for four days at 37°C in the presence or absence of Vitamin D.

Results: Proteomic analysis showed increase expression of enzymes involved in the glutaminolysis process, such as glutaminase (GLS), glutamate dehydrogenase (GDH), and mitochondrial aspartate aminotransferase (GOT2), when T-cells were cultured in the presence of Vitamin D. In addition, enzymes associated with glycolysis were reduced by Vitamin D. Then, viability, cell counts, and glycolytic parameters were evaluated. A decrease in glucose uptake and lactate production, alongside increases viability and cell counts were observed with Vitamin D. Then, the inhibitor of the glutamine transporter (ASCT2) (GPNA) and two glutaminase inhibitors, BPTES and 968, decreased T-cell counts induced by the presence of Vitamin D.

Conclusion: These results suggest that the glutaminolytic pathway is induced by Vitamin D and plays a crucial role at maintaining $CD4^{+}$T-cell counts, opening the door for further study of this metabolic pathway.
Financing: VRID Nº2023000835.

P-46

Potential prediction of glioblastoma stem-like cells subtype through detection of miR-155-5p contained in extracellular vesicles from liquid biopsies

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Introduction: Treatment failure in Glioblastoma (GB) is mainly caused by a subpopulation of "Glioblastoma Stem-like-Cells" (GSCs). There are two subtypes of GSCs: Mesenchymal (GSC-MES) and Proneural (GSC-PN), with GSC-MES being the ones that are associated with a more aggressive phenotype, where the detection of biomarkers for their identification becomes relevant. Liquid biopsies are one of the minimally invasive strategies to detect biomarkers, in particular, extracellular vesicles (EVs). EVs contain different macromolecules inside, such as microRNAs (miRs). miR-155-5p is enriched in EVs from GSCs and is associated with therapeutic resistance.
Objective: To detect the levels of miR-155-5p in EVs from plasma of patients with GB to identify the GSC-MES subtype.
Methods: miR-155-5p levels were measured by RT-qPCR from cell culture samples of GSCs subtypes and in plasma EVs from GB patients (approved on 02/06/21 by the SEC of the S.M.M.O). The data obtained were compared with the characterization of the cultures by qPCR and flow cytometry.
Results: We found increased levels of miR-155-5p in GSC-MES (15,99 ± S.D.=2,50; n=2) vs GSC-PN (0,66 ± S.D.= 0,36; n=3). Likewise, we found higher levels of miR-155-5p in patients with GB (5,87 ± S.D.=2,74; n=7) vs. healthy controls (0,29 ± S.D.=0,47; n=4). Statistical analyzes were performed using T-student.
Conclusion: This information suggests that miR-155-5p is a potential biomarker to identify GSCs subtypes from plasma and can be used as a future biomarker for monitoring during treatment as well as to find a targeted therapy and deliver a better life expectancy to the GB patient.
Financing: Fondecyt Nº 1200885, Instituto Milenio de Inmunología e Inmunoterapia (IMII).

AREA: LIPIDS AND NUTRITION

P-47 ★*selected for oral communication*

Docosahexaenoic acid (DHA) supplementation during pregnancy increases the expression of placental antioxidant enzymes in pregnant women with Gestational Diabetes Mellitus

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Introduction: Introduction: Gestational diabetes mellitus (GDM) is a glucose intolerance diagnosed for the first time during pregnancy and resolves after delivery. It has been shown that GDM is associated with increased oxidative stress and a

decrease in the antioxidant capacity in the placenta. Limited data shows the potential antioxidant effect of Docosahexaenoic acid (DHA) during pregnancy. However, the effect of DHA on the antioxidant capacity of the GDM placenta is unknown.

Objective: To evaluate the effect of maternal DHA supplementation on placental antioxidant enzyme expression in pregnant women with/without GDM.

Methods: This is a secondary study of a double-blind randomized trial of maternal DHA supplementation during pregnancy with a normative dose (200 mg/d) or 800 mg/d DHA (ethical approval ID-160825023). We analyzed the expression of the genes of the antioxidant enzymes Superoxide Dismutase1 (SOD1), Superoxide Dismutase2 (SOD2) and Glutathione Peroxidase1 (GPX1) (RT-qPCR) in trophoblast from women c/GDM800-(n=8), c/GDM200-(n=7) and s/GDM800-(n=8), s/GDM200-(n=12) who participated in this RCT. Statistics: Mann-Whitney test for group comparisons ($p<0.05$).

Results: In the GDM placenta, a reduced expression of SOD1 ($p=0.037$) and GPX1 ($p=0.054$), with no changes in SOD2, was observed. Maternal DHA supplementation (800 mg/d) increased SOD2 in non-GDM and GPX1 in GDM women, without changes in SOD1 expression.

Conclusion: These results suggest that DHA supplementation in women with high-risk of GDM could be a nutritional strategy to prevent the effects of early maternal and fetal exposure to a prooxidant milieu. Oxidative stress markers in proteins and lipids of blood samples of these patients are under analysis.

Financing: Fondecyt 1221812Fondecyt 1220549SOCHINUT.

P-48 ★*selected for oral communication*

Deleterious effects of high-fructose diets in BALB/c mice

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Introduction: The increased consumption of fructose is associated with the use of high-fructose corn syrup in ultra-processed foods as well as to the development of fatty liver, obesity and diabetes.

Objective: To investigate the effects of chronic consumption of high-fructose diets on the intestinal mucosa of male BALB/c mice.

Methods: Fructose was supplemented in normal diets at 10%, 30% and 50% diet for 1, 2 and 4 months (n=4 in each group). We recorded body weight, gonadal fat weight, plasmatic parameters (glucose, fructose, lipids), redox state of the intestinal mucosa (lipoperoxidation, protein carbonylation, catalase activity and redox enzymes expression) as well as the expression of fructose transporters GLUT5-8-12 in intestinal mucosa. The protocols were approved by the Institutional Ethic Committee and the data was expressed as mean ± standard deviation. ANOVA test was used to statistical analysis.

Results: Diets F30 and F50 produced significant changes in glycemia at 2 and 4 months ($p>0.01$) as well as in fructosemia, gonadal fat tissue, body weight gain, triglycerides, total cholesterol and HDLc ($p<0.05$) at 4 months. In the intestinal mucosa at 2 months F30 increased lipid peroxidation by 2.2-fold ($p<0.05$), while F50 decreased catalase activity 80% ($p<0.05$). The expression of fructose transporters depended on the duration and dosage of the diet, with 30-fold increase in GLUT8 at 2 months in the F50 group.

Conclusion: The systemic and intestinal effects of high-fructose diets were dependent on the duration and dosage of the diet and could contribute to the development of obesity and diabetes.

Financing: DI-REG 11/2021, CIBAS-UCSC.

AREA: NEUROPHYSIOLOGY

P-49

Role of dynamin´s GTP-ase activity on the dendritic spine morphogenesis in hipocampal neurons

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Introduction: The cytoskeleton is a polymer-network, critical for the formation of neuronal sub-structures such as axons, dendrites and spines. These latter are dendritic protrusions that constitute the main site of synaptic contact between neurons in the mammalian brain. The number and size of spines determine changes in the neurotransmission´s efficiency, a phenomenon known as synaptic plasticity. In neurological contexts, the loss of neuronal morphology occurs, in a way dependent on cytoskeleton defects. Therefore, intervening cytoskeleton could be a promising strategy to prevent the loss of neuronal sub-structures such as dendritic spines.

Objective: To evaluate the role of dynamins, an actin/microtubule-modulator, on the morphogenesis of dendritic-spines.

Methods: Hipocampal neurons were cultured from postnatal mice following approved bioethical guidelines(BEA-192-23). 10-days neurons were treated with 20 uMBIST23, a dynamin promoter, or with the vehicle DMSO, upon resting conditions or upon induction of long-term-potentiation(LTP). Neurons were fixed, stained with phalloidin and visualized by confocal microscopy. Spine density and size were analyzed using the ImageJ-software. Data were analyzed by a-one-way-ANOVA-test and expressed as mean ± SEM. N= 3 to 15 neurons per experimental condition. $P<0.05$ was considered significant.

Results: The LTP-induction enhanced the size and number of dendritic spines compared to resting-neurons. The treatment with BIST23 had similar effects, increasing spine-density and size compared to DMSO-treated neurons.

Conclusion: These preliminary results suggest that enhancing dynamin´s activity could be considered as a potential alternative to promote dendritic spine morphogenesis, avoiding the loss neuronal sub-structures in pathophysiological conditions**.**

Financing: This work has been funded by Fondecyt 1231511.

P-50

Serotonin-endocannabinoid crosstalk induces selective long-term depression at GABAergic inhibitory synapses in the medial prefrontal cortex

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Introduction: Serotonergic (5-HT) fibers from the raphe nuclei are known to regulate neuronal excitability and glutamatergic synaptic function in the prefrontal cortex (PFC) by activating 5-HT2a receptor subtype (5-HT2aR). However, little is known about the mechanisms by which 5-HT2aR tune inhibitory synaptic strength in mPFC.

Objective: We challenged the hypothesis that 5-HT2aR activation evokes a long-lasting change in inhibitory synaptic strength in mPFC pyramidal neurons.

Methods: GABAergic inputs onto layer II/III pyramidal neurons were electrically stimulated while various pharmacological manipulations were performed. Somatostatin-cre, Parvalbumin-cre and Fev-cre animals were intracranially injected with a channelrhodopsin-2 into mPFC and dorsal raphe nuclei. We performed t-student test or ANOVA coupled to *post-hoc* test. Significance was set at $p < 0.05$ (*). All procedures outlined in this study were approved by the University of Valparaiso Bioethics committee (BEA136-19).

Results: We find that brief pharmacological activation of 5-HT2aRs induces a long-term depression of electrically-evoked inhibitory postsynaptic currents (IPSC-LTD). 5-HT2aR-mediated IPSC-LTD requires the activation of type 1 cannabinoid receptors (CB1Rs). We hypothesize that 5HT2aR activation may trigger endocannabinoid production to recruit presynaptic CB1Rs to subsequently suppress GABA release. Notably, repetitive optogenetic activation of 5-HT fibers alone is sufficient to trigger IPSC-LTD and requires both 5-HT2aRs and CB1Rs activation. Interestingly, 5-HT2aR- and CB1R-mediated IPSC/LTD is input specific, occurring at inhibitory

synapses from somatostatin- but not parvalbumin-positive GABAergic interneurons.
Conclusion: Our findings reveal a novel form of 5-HT-mediated regulation of GABAergic synaptic strength that is input-specific and strongly support a crosstalk between 5-HT2aRs and CB1Rs to modulate GABAergic inhibition in the mPFC.
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P-51

A physiological pain model of fibromyalgia induced by neonatal stress in rats

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Introduction: Fibromyalgia (FM) is a syndrome marked by widespread musculoskeletal pain without an injury or peripheral damage, making its physiological etiology unclear and challenging for standardization. To be considered as an FM model, it should replicate FM symptoms, pharmacological responses, triggered by physiological stimuli, and display gender preference.
Objective: Characterize the FM model in rats through neonatal stress via limited nesting material.
Methods: Neonatal stress is induced in female Sprague Dawley rats and offspring by limiting nesting (neonatal limited bedding-NLB) between days 2-9 postpartum, placing them in cages with only thin paper strips, meanwhile controls had conventional cages. Hyperalgesia is measured in NLB-offspring once reach 200-300g using the Randall Selitto method, and characterized by the analgesic effect of duloxetine(60 mg/kg) or gabapentin(100mg/kg). In addition, changes in inhibitory control were studied by electromyographic activity in the gastrocnemius muscle through C-fiber reflex generated by electrical stimuli in the hind paw. Results presented in±S.E.M. Statistical analysis was done using ANOVA, Tukey post-test, or t-test with p-value <0.05. USACH Scientific Ethical Committee approved this protocol under the N°368.2022.
Results: NLB-rats showed significant hyperalgesia: males 126±3g/cm^2(n=19), females 109±2g/cm^2(n=21), compared to controls: males 229±6g/cm^2(n=14), females 197±7g/cm^2(n=12). Hyperalgesia reduced in FM rats post duloxetine 60 mg/kg or gabapentin 100mg/kg treatment, achieving paw thresholds: males 198±6g/cm^2(n=6), females 191±10g/cm^2(n=6) and 221±11g/cm^2(n=6) to 252±8g/cm^2(n=6) respectively. The control group showed male and female electromyographic activity reduction: 54±4%(n=6) and 55±8%(n=6). Besides NLB males had 22±7%(n=11) reduction, females 41±4%(n=10), indicating DNIC alteration in NLB rats.
Conclusion: Neonatal stress provides a robust FM model for adult rats.
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Probenecid oral administration prevents spatial memory and synaptic defects in a mouse model of Alzheimer's disease

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Introduction: Probenecid (PBN) is a drug used for gout treatment to reduce the renal excretion of antibiotics. Evidence has also shown a neuroprotective effect in several conditions affecting the central nervous system. Furthermore, PBN is a known blocker for Pannexin-1(Panx-1) channels, a membrane protein that modulates the induction of excitatory synaptic plasticity under physiological contexts and it contributes to neuronal death under inflammatory conditions, such as Alzheimer's disease (AD), condition where this protein is increased in mice models.

Objective: We investigated the impact of the treatment with PBN on the Panx1 channel activity, synaptic structure, and cognitive function in a murine model of AD.

Methods: We treated during one month with PBN, 3, 12, and 18 m.o APP/PS1 and wildtype mice. The cognitive function was evaluated by Open field, Novel Object recognition, and Morris Water Maze tests. Histological, biochemical, and electrophysiological studies were realized to evidence morphological and functional changes in the synaptic structures and the Panx1 channel activity. Values are presented as mean±SEM. N=3-4 animals/group. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison post-test. All animal manipulations were approved by the Ethics and Animal Care Committee of Universidad de Valparaíso (BEA160-20).

Results: The treatment with PBN prevented the defects in recognition and spatial memory, observed in 12 m.o APP/PS1 mice, and also increased dendritic arborization and spine density in WT and APP/PS1 mice. Furthermore, PBN prevented synaptic plasticity defects in APP/PS1 mice.

Conclusions: These findings suggest that a "neuroprotective" effect of PBN could rely on the blockade of Panx1 channels.

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Modulation of brain endothelial cells and microglial interaction through the TNF-α/Panx1 signaling pathway

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Introduction: Pannexin 1 (Panx1) is a membrane channel involved in ATP release and the nervous system's various physiological and pathophysiological functions. Has been postulated that TNF-α activates Panx1, increasing extracellular ATP release from endothelial cells, leading to microglial migration and accumulation around cerebral blood vessels through purinergic signaling. Therefore, microglia activation is through purines released through Panx1 endothelial channels.

Objective: In this study, using an *in vitro* inflammatory model, we investigated the impact of TNF-α on the Panx1, which modulates the interaction between microglia and endothelial cells under inflammatory conditions.

Methods: To address this, we treated cultured mouse brain endothelial cells (BECs) with TNF-α (10 ng/ml) for 1, 5, and 24 h to induce an inflammation model in the presence of Panx1 blockers. Following this, we measured by immunostaining and immunoblot the presence of proteins like CD-31, Panx1, Panx-1Y198. Next, we used the co-culture of BECs and microglia to measure the migration. All animal manipulations were approved by the Ethics and Animal Care

Committee of Universidad de Valparaíso (BEA160-20).

Results: TNF-α effectively governs both the overall transcription of Panx1 (0.957±0.2705, n=11 vs.1.959±1.002, n=9) and the post-translational modification of Panx-1Y198 (0.3012±1.559, n=3 vs.8.755±8.453, n=3). in BECs. Interestingly, microglial migration towards BECs becomes negligible after applying a Panx1 channel blocker (0.000±0.000, n=7 vs.11.50±2.110, n=5).

Conclusion: Our results suggest that an *in vitro* inflammation model by TNF-α modulates the interaction between BECs and microglia by Panx1 channels. Therefore, we can assert that Panx1 significantly modulates the interaction between microglia and endothelial cells under inflammatory conditions.

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Gliotransmitters interplay in brainstem astrocytes cultures

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Introduction: Central respiratory chemoreception is fundamental for the generation and modulation of the respiratory rhythm, allowing breathing to be adjusted to the physiological demands. Astrocytes secrete ATP, glutamate, and D-serine in response to hypercapnia, driving the central chemoreflex.

Objective: To evaluate ATP, glutamate, and D-serine interactions in astrocyte cultures.

Methods: Intracellular calcium was determined in cultures of brainstem astrocytes loaded with Oregon Green Bapta-1 (n=60 cells recorded from n=4 independent cultures). Superfusion with aCSF containing D-serine (50 µM) or glutamate (50 µM) or ATP (20 µM) was performed in cultures incubated with purinergic receptor blockers or DAAO. We also studied the time course of the concentration of ATP and D-serine during hypercapnia using HPLC detection. Ca+2i and HPLC data were analyzed by paired t-test, and unpaired t-test, respectively, the values were represented as mean±SD. USACH's Bioethics Committee approval code is 180/2021.

Results: Elimination of extracellular D-serine with DAAO, the enzyme that degrades it, increases ATP-induced [Ca+2]i increase from 10%±0.53 (ATP) to 24%±0.84 (ATP+DAAO) over basal level (p=0.001). Additionally, the blockade of P2Y receptors reduces the glutamate-induced increase in [Ca+2]i from 5.2%±0.2 (glutamate) to 4.6% ± 0.3 (glutamate+suramine) over basal level(p=0.0115). The time course of ATP and D-serine secretions in astrocyte cultures show a peak between 60 and 120 s of the beginning of hypercapnic stimulus.

Conclusion: Our results show that the magnitude of ATP and D-Serine evoked effects depend on the presence of each other. During hypercapnia, both are released with similar time courses allowing the maximal interaction between them.

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Intranasal vasopressin administration reduces amphetamine relapse in adolescent male rats

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Introduction: Adolescence is a vulnerable period for drug abuse and drug dependence or addiction development. Amphetamine (AMPH) is a psychoactive substance commonly used as a recreational drug by young people, and there is a lack of effective medications for the treatment of AMPH or other psychostimulant addiction. Recent studies have shown that the vasopressin (AVP) system plays a significant role in drug addiction, making it an interesting therapeutic target.

Objective: This research aims to study the effect of intranasal AVP administration during the

conditioned place preference (CPP) extinction phase on AMPH-induced reinstatement.

Methods: Male and female adolescent Sprague-Dawley rats (43-45 postnatal days) were conditioned with AMPH (1.5 mg/kg i.p.) or saline for 5 days. Subsequently, during 5 days of extinction, the animals received intranasal treatment with AVP (20 µg/kg) or saline. Following the extinction phase, reinstatement was induced by an AMPH injection (bioethics N°383/2022). Glutamate, GABA, and dopamine levels were quantified in the reward system's nucleus. Two-way ANOVA followed by Fisher post-hoc test (CPP to AMPH) and Mann-Whitney were used. The data are expressed as mean ± S.E.M.; n = 5.

Results: The results suggest that intranasal AVP administration during the extinction phase partially blocks CPP reinstatement or drug-seeking behavior in male rats only ($t(36)=1.757$, $p=0.0874$), increasing glutamate and GABA tissue levels in the ventral tegmental area ($U=2$, $p=0.032$, $n=5$; $U=1$, $p=0.016$, $n=5$).

Conclusion: In males, AVP may inhibit glutamate release in the VTA through increased GABAergic tone of VTA interneurons decreasing drug reinstatement.

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Chaotic physiological synchrony during collaborative emotive recall

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Introduction: Nonlinear physiological synchrony was assessed, and its implications for dyadic interaction during a collaborative recall task were explored.

Methods and Results: Eighty-two young individuals (average age 21.1 ± 3.56 years) were engaged in pairs, capturing physiological data during a joint narrative recall task, segmented into 21 dyads with affective elements and 20 dyads without. The study employed physiological synchrony metrics for analysis. The research emphasized unique trends in physiological synchrony between emotional and non-emotional situations, noting increased synchronization in emotional settings. Crucially, the Lyapunov Coefficient, in conjunction with the Cross Correlation and Coherence indices, was essential in identifying emotional conditions within collaborative settings, highlighting the significant contribution of the Lyapunov Coefficient in studying emotional involvement in group memory activities.

P-57

Brainstem localization of serine racemase suggests different loci for ATP/D-serine interactions

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Introduction: Central chemoreception is a main sensory modality regulating breathing. Astrocytes, in response to increased CO_2/H^+ levels, can release ATP or D-serine, which in turn, activate the respiratory neural network. There is a controversy about the cells that synthetize D-serine.

Objective: Here, we study the cellular location of serine racemase in brainstem and addressed whether the respiratory responses induced by D-serine on brainstem slices depend on purinergic receptor activation.

Methods: Immunodetection of D-serine racemase was performed in CF1 adult mice (USACH's Bioethics Committee approved the study with the code 180/2021) using immunofluorescence and confocal microscopy (preliminary results). Fictive respiration was recorded with glass suction electrodes in caudal brainstem slices from CF1 mice (P1-P7), superfused with aCSF equilibrated with O2/CO2 = 95%/5%, (pH 7.4, 30 °C). Concentration-response curves for the increase in respiratory frequency (f_R) induced by D-serine (0.5-100 µM) were performed in the presence and absence of purinergic blockers, MRS2179 or suramin. Differences between concentration-response curves were analyzed with a two-way

ANOVA test followed by a post hoc test (mean ± SEM; n=6 control vs each purinergic blocker). **Results:** D-serine racemase could be localized in a fraction of astrocytes and microglia, being more abundant in neurons at caudal medullary areas. MRS2179 or suramin superfusion reduced the D-serine-induced increase of f_R.
Conclusion: Our results indicate that the respiratory effect of D-serine depends on the activation of ATP receptors, suggesting a functional interaction between both gliotransmitters. The broad cellular expression of serine racemase suggests different sites for interaction between ATP and D-serine.
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P-58

Metabolic and functional assessment of the olfactory bulb and hippocampus in a model of metabolic syndrome induced by a high sucrose and a high fat diet in rats

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Introduction: Insulin resistance and oxidative stress, major components of metabolic syndrome (MetS) are involved in amyloid beta build up, linking MetS to neurodegeneration and early mental diseases, depending on MetS severity.
Objective: To evaluate the effect of two hyper-caloric diets, representing early and severe MetS stages, on olfaction, memory, antioxidant systems and amyloidogenic and insulin pathways in the hippocampus and olfactory bulb in rats.
Methods: Male Wistar rats (250 g) received a high-sucrose diet, early MetS, (HSD; n=5) for 24 weeks or a high-fat diet, severe MetS, (HFD n=9) for 16 weeks, control groups received standard diet. Two weeks before diet completion, open field test was used to discard anxiety or mobility impairment. Olfaction was assessed by buried-food and odor habituation-dishabituation tests. Memory was evaluated by novel object location and Barnes maze. Antioxidant enzyme activity and expression of BACE1, APP, AKT, AKT-P and insulin receptor were quantified in tissue homogenates. Values are mean ± S.E.M. Differences were determined by Mann-Whitney test. Protocol was approved by institutional scientific committees (INP 056/2022).
Results: MetS induced by any diet did not produce anxiety, impaired mobility or long-term spatial memory. HFD deteriorated social odor perception and impaired short-term spatial memory. The hippocampus from HSD rats revealed decreased expression of amyloidogenic proteins, hyperactivation of the insulin pathway, low levels of lipoperoxidation, and increased activity of antioxidant enzymes.
Conclusion: HFD affected olfaction and memory. Molecular data show that HSD may undergo a compensatory effect in brain insulin resistance and antioxidant activity, probably preceding memory lost.
Financing: This work is partially funded by INP Federal Program E022.
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Hunger enhances hippocampal memory consolidation during wakefulness

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Introduction: It has been widely accepted that sleep is necessary for memory consolidation. However, recent evidence indicates that fasting may potentiate memory consolidation even without a sleep period after learning.
Objective: This work aims to evaluate the ability of rats to consolidate hippocampal-dependent memories after fasting in the absence of sleep.
Methods: Seven adult male Sprague Dawley rats under standard light-dark cycle conditions were evaluated for their performance on the Object in Place Recognition test under ad libitum, 24-h and 48-h fasting conditions while they were either sleep deprived or not after learning (Bioethics approved protocol number:1158). Statistically significant differences were assessed using a two-way RM ANOVA followed by a Holm-Sidak Post

hoc test. All data are represented as mean ± SEM. **Results:** The memory performance of the sleep-deprived animals in the ad-libitum condition showed a significant decrease compared with those in the ad-libitum condition but allowed to sleep. Interestingly, the memory performance of 48 hours of fasting and sleep-deprived rats showed memory performance very similar to control (ad libitum/ non-sleep deprived) rats. Furthermore, these results are not explained by differences in exploration time or traveling distances, discarding any movement-derived effects induced by experimental manipulations.
Conclusion: These results suggest that under fasting conditions, hippocampal memory consolidation may occur in the absence of sleep. Also, these results are a starting point to explore the physiological mechanisms involved in hippocampus-dependent memory consolidation during wakefulness in adverse survival conditions such as food scarcity.
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Impact of Bis-T-23, an enhancer of dynamin´s activity, on the synaptic availability of AMPAR in hippocampal neurons from APP/PS1 mice, a murine model of Alzheimer Disease

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Introduction*:* Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by a decrease in the cognitive functions. An early pathological mechanism is a decrease in the availability of the glutamatergic AMPA-receptor in the postsynaptic densities, leading to impaired glutamatergic neurotransmission. A modulator of the AMPAR trafficking are dynamins. These are GTP-ases that promote AMPAR insertion and endocytic recycling to and from PSDs. Therefore, it is feasible that enhancing dynamin´s activity could increase the synaptic AMPAR availability.
Objective: To evaluate the impact of Bis-T-23, a dynamin enhancer, on the synapse formation and AMPAR PSD-availability in hippocampal neurons cultured from APP/PS1 mice, a murine model of AD.
Methods: Approximately 228 hippocampal neurons cultured from postnatal (P0-P2) APP/PS1 and wildtype (WT) mice were treated at 10 days in vitro with Bis-T-23, or the vehicle DMSO. Neurons were fixed, immunolabeled with antibodies against GluR1-AMPAR-subunit, PSD95 or synaptophysin (SYPH) as post- and pre-synaptic markers, respectively. SYPH/PSD95 and GluR1/PSD95 colocalization (as PCC) was analyzed to evaluate synapse formation and synaptic-AMPAR availability, respectively. Experiments were performed under resting conditions and under chemical induction of Long-term-potentiation. Data were analyzed by a one-way-ANOVA parametric test and Tukey's multiple comparison test, $p<0.05$ was considered significant. The protocols were approved by the CICUAL with act BEA 011-2023.
Results*:* APP/PS1 neurons exhibited reduced synapse-formation and AMPAR-availability compared to WT upon both, resting and LTP-conditions. The treatment with Bis-T-23 significantly prevented such defects in LTP-APP/PS1 neurons.
Conclusion*:* Increasing dynamin GTP-ase-activity with Bis-T-23, could represent a promising strategy to prevent synaptic defects in the context of AD.
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Effect of BIST23, a promoter of dynamins, on the density and size of dendritic spines in hippocampal neurons of a murine model of Alzheimer's Disease

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Introduction: Alzheimer's disease (AD) is a leading cause of dementia in elderly. One of the earliest events in AD, that correlates with the onset of the cognitive decline, is the loss of functional synapses and dendritic spines. These are actin-enriched dendritic protrusions where most of the synaptic contacts occur. Dendritic-spine loss correlates with an imbalance in the actin cytoskeleton dynamics, leading to synaptic defects in AD. A decrease in dynamin´s expresión, GTPases that regulate actin dynamics, has been reported in *postmortem* brains of AD-patients, suggesting that enhancing dynamins´s activity could be a strategy to intervene the synapse-loss in AD.
Objective: To evaluate the effect of BIST-23, an enhancer of dynamins´s activity, on the spine density and size in hippocampal neurons cultured from APP/PS1 mice, a murine model of AD.
Methods: Hippocampal neurons cultured from postnatal APP/PS1 and wildtype (WT) mice were treated with 20 uM BisT23, or its vehicle DMSO, then fixed, stained with phalloidin and visualized by confocal microscopy. Spine morphometric analysis was performed with the software ImageJ. Experiments were performed under resting conditions and upon chemical induction of Long-term-potentiation (LTP). Data were analyzed by a one-way-ANOVA-test and expressed as mean ± SEM. N= 7 to 16 neurons per experimental condition. Bioethics Committee approval with the code BEA 009-2023.
Results: APP/PS1 neurons exhibited reduced spine density at rest and LTP-condition. BIST23 significantly prevented this defect. The spine-size was unchanged between experimental conditions.
Conclusion: These data strongly suggest that dynamin´s potentiation could prevent the loss of dendritic spines in AD.
Financing: This work was funded by FONDECYT 1231511, Dra. Arlek Gonzalez Jamett

AREA: RENAL AND GASTROINTESTINAL

P-62
Study of the A2BAR in profibrotic activation of glomerular parietal epithelial cells in experimental diabetes

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Introduction: Diabetic nephropathy (DN) is a complication of diabetes characterized by the loss of podocytes which results in the development of proteinuria and glomerulosclerosis. Podocytes are terminally differentiated cells without the capacity to selfrenew and while the parietal epithelial cells (PEC) of the Bowman's capsule have been identified as podocyte progenitors, however some studies have shown that in DN, PEC cells undergo profibrotic activation. The progression of DN has been correlated with high levels of adenosine. Because we have determined that the antagonism of adenosine receptor A2B (A2BAR) attenuates fibrosis and proteinuria in experimental DN, this study addresses role of A2BAR on the profibrotic activation of PEC.
Methods: The use of animals for this project was approved by Bioethic Committee nº 465/2022. Experimental diabetes was induced in rats using streptozotocin. Following one month after diabetes induction, the A2BAR antagonist MRS1754 (0.5mg/kg/48h) was administered to diabetic rats for 4 weeks. The PEC CD24+CD56+ subpopulation and PEC CD9+CD44+αSMA+ cells undergoing profibrotic activation were analyzed through immunohistochemistry and western blot. The statistical analysis utilized was Student *t* test for 3 individual samples.
Results: We determined a decrease in CD24 and CD56 levels and an increase of the profibrotic markers CD9, CD44 and αSMA in the bowman´s capsule of diabetic rats. The use of A2BAR receptor antagonist MRS1754 in diabetic rats attenuates the increase of CD9, CD44 and αSMA and increase the levels of CD56.
Conclusions: The antagonism of A2AR blocks the profibrotic activation of PEC, which may preserve their potential to differentiate toward podocyte.
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Unilateral ureteral obstruction is associated with a downregulation of the NGAL receptor in renal

medulla and with a modification in cellular distribution at renal cortex level

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Introduction: The inflammatory process is critical for the development and progression of chronic kidney disease (CKD). Previously, we have demonstrated that Neutrophil Gelatinase-Associated Lipocalin (NGAL), a 25kDa glycoprotein secreted by different tissues, promotes an inflammatory phenotype in kidney and is necessary for fibrosis progression in experimental models of CKD. However, the renal NGAL receptor (24p3R), which is mainly expressed in distal tubule has been poorly studied in CKD.

Objective: To determine the renal expression and distribution of 24p3R in an experimental inflammatory model of CKD.

Methods: Male C57BL/6J Wild-Type mice (8-12 weeks, n=8) were undergone to unilateral ureteral obstruction (UUO) or Sham surgery (control) for 7-days (bioethical approval #0828FMUCH). In addition to histological and functional analyses, we studied the renal abundance/distribution of 24p3R by qRT-PCR/Western-Blot and Immunohistochemistry, respectively. Data are presented as mean ± S.E.M., and Mann-Whitney test were performed.

Results: UUO induced tubular dilation (P<0.001), without changes in creatininemia neither blood urea nitrogen levels. Basement membranes thickening in UUO kidney was observed, which was in accordance with an increase of NGAL in urine and plasma (134.5hg/mL, P<0.05). At cortical level, non-differences in the abundance of 24p3R were observed after UUO. However, we observed a predominantly cytosolic distribution in UUO tubules, which differed from the apical distribution in control kidney. In renal medulla, we observed a decrease in 24p3R abundance in UUO group (P<0.05).

Conclusion: The 24p3R undergoes to a significant down-regulation during the inflammatory kidney damage, which coincide with an increase in NGAL suggesting a negative feedback interaction.

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The modulation/increase of Na+/K+-ATPase activity prevents Ischemia-Reperfusion Injury (IRI)-induced Acute Kidney Injury (AKI)

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Introduction: Ischemia induces Na^+/K^+-ATPase dysfunction due to insufficient ATP supply, resulting in subsequent cellular damage. We developed a novel 4th generation of SMEF (Synchronization Modulation Electric Field; 4th-SMEF) incorporating dual-energy transformation functions and power injections for ATP generation that efficiently controls Na^+/K^+-ATPase activity during ischemia.

Methods: We tested the effects of the 4th-SMEF in an ischemia reperfusion-induced acute kidney injury (IRI- AKI) model in C57BL/6J mice (male and female). The animal use adhered to NIH Guide for the Care and Use of Laboratory Animals, following protocols approved by USF IACUC. After right kidney nephrectomy, AKI was induced by clamping of the left renal pedicle for 20 min in male and 25 min in female mice.

Results: Application of the 4th-SMEF to the kidney during clamping reduced plasma creatinine (injury marker) levels by 92% in males (AKI: 2.56±0.9 mg/dL; AKI with 4th-SMEF: 0.21±0.10 mg/dL, n=5) and 82% in females (AKI: 1.84±0.4 mg/dL, AKI with 4th-SMEF: 0.34±0.3 mg/dL, n=5). Glomerular Filtration Rate was improved by 60% in males (AKI with 4th-SMEF: 255 µl/min, AKI: 110 µl/min) and 55% in females (AKI with 4th-SMEF: 220 µl/min, AKI: 98 µl/min). Na^+/K^+-ATPase expression levels evaluated by WB analysis were significantly reduced in AKI groups and restored almost to control levels after application of the 4th-SMEF. The 4th-SMEF also almost reduced the levels of kidney injury evaluated by KIM-1, N-Gal, and histology (H&E and PAS staining) in the AKI group to similar levels to the controls.

Conclusion: 4th-SMEF prevented IRI-induced AKI by normalizing Na+/K+ pump activity.

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P-65

Role of the (pro)renin receptor (PRR) on the expression of profibrotic markers in primary

cultures of mouse renal collecting duct cells during high glucose conditions

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Introduction: Diabetes mellitus (DM) is a metabolic disorder that leads to kidney function decline. The renin-angiotensin system (RAS) regulates body fluid balance and blood pressure. In DM, inhibiting RAS has beneficial effects on renal functions, suggesting its involvement. Additionally, pro-hormone prorenin increases in plasma and is overexpressed in renal collecting tubules in diabetes. The (pro)renin receptor (PRR) is a RAS component and is overexpressed in diabetic animal models. While in vivo evidence supports PRR induction in DM, it's unclear if high glucose conditions increase PRR in primary cultured collecting duct cells (IMCD).

Objective: To determine the effect of incubation with high glucose and PRR inhibition on NOX-4, TGFβ, fibronectin, and CTGF expression in the IMCD.

Methods: IMCD cell cultures were made from inner medullary tissues (Bioethical Committee N° BIOEPUCV-B 267-2019). We measured renin in a culture medium and used the PRR antagonist peptide "PRO20" under a 48-hour normal-glucose (NG) condition (5,5 mM) and high-glucose (HG) condition (25 mM) to assess the profibrotic factor protein expression through Western blot. Results are presented as mean ± SEM, n=4. We evaluated the normal distribution of each analyzed parameter using the Shapiro-Wilk test. One-way ANOVA compared mean differences between groups.

Results: High glucose treatment increased renin protein levels in the IMCD culture medium, and it upregulated protein levels of NOX-4, TGFβ, and CTGF in cell lysates. Pre-incubation with PRO20 prevented the increase in NOX-4 and TGFβ. However, this effect did not extend to fibronectin and CTGF.

Conclusion: These findings help clarify PRR's role in explaining tubular-level fibrosis development due to chronic hyperglycemia.

Financing: FONDECYT 1220525.

P-66

The absence of the oxoglutarate receptor 1 (OXGR1) prevents the increases in blood pressure and modulates the expression of the (pro)renin receptor in the kidneys of mice with two-kidney - one clip model (2K1C)

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Introduction: Reduced kidney blood perfusion triggers activation of renin-angiotensin system (RAS), which induces vasoconstriction and sodium retention. The (pro)renin receptor (PRR) plays a significant role in intrarenal RAS activation in the renal collecting duct (CD). Reduced renal blood flow causes dysregulation of Krebs cycle, augmenting α-ketoglutarate in the kidney. Previous reports showed that α-ketoglutarate Receptor 1 (OXGR1) present in CD enhances PRR expression. We hypothesize that the absence of OXGR1 prevents PRR expression, disrupting RAS activation and blood pressure (BP).

Objective: To investigate the effect of reduced renal blood flow induced by Goldblatt model (2K1C) on BP and renal PRR protein levels in OXGR1 knockout mice.

Methods: In C57BL/6 wild type or OXGR1$^{-/-}$ knockout mice the renal artery of the left kidney was clipped (0.13 mm; n=4). A sham procedure was applied in control mice (n=6; bioethical committee Nº BIOEPUCV-BA 482-2022). After 2 weeks, 2K1C surgery was validated by the reduced clipped kidney size. Values are reported as mean ± S.D and were analyzed using ANOVA.

Results: Increases in systolic BP were observed in wild type mice (2K1C: 82 ± 5.254 mmHg / Sham: 101.8 ± 6.801 mmHg, $p<0.05$). In OXGR1 knockout mice the increase wasn't significant (2K1C KO: 92 ± 12.99 mmHg). PRR protein levels were augmented in clipped kidneys (fold change: 1.72 ± 0.05 / 1.00 ± 0.14, $p<0.05$), but not in OXGR1 knockout mice (1.11 ± 0.16-fold change, $p=0.61$).

Conclusion: Our results provide insights into OXGR1-dependent PRR regulation during reducing renal blood flow impacting on blood pressure.

Financing: FONDECYT 1220525

P-67

Pharmacological blockade of the Oxoglutarate Receptor 1 (OXGR1) prevents the upregulation of

the (Pro)renin Receptor and attenuates the increases in blood pressure in mice with Two-Kidney One-Clip Model (2K1C)

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Introduction: Reduced blood perfusion to the kidney activates the renin angiotensin system (RAS) causing sodium retention impacting on the arterial blood pressure (BP). The α-ketoglutarate Receptor 1 (OXGR1), has been identified in the collecting ducts, a site that expresses the (pro)renin receptor (PRR) which is able to increase intratubular RAS activity increasing BP. We showed that OXGR1 enhances PRR expression.

Objective: to test the effect of the reduced renal blood flow (Goldblatt model 2K1C), on PRR protein expression in renal medullary tissues and the effect in BP in the presence or absence of OXGR1 antagonist montelukast.

Methods: In C57BL/6 mice the renal artery of the left kidney was clipped (0.13 mm), n=6. A sham procedure was applied in control mice (n=6, bioethical committee Nº BIOEPUCV-BA 482-2022). Similarly, other group was infused with or without montelukast at 5 mg/kg/day. After 2 weeks, 2K1C surgery was validated by the evidence of the reduced clipped kidney size. Values are presented as mean ± S.D and analysis done with ANOVA.

Results: Significant increases in systolic BP were observed in wild type mice after 2 weeks (2K1C: 101.8 ± 6.801 mmHg vs. Sham 82.0 ± 5.254 mmHg, $p<0.05$), Montelukast prevented this increase (2K1C + ML: 90.5 ± 7.503 mmHg). PRR expression was augmented in clipped kidneys (1.76 ± 0.05-fold change of control $p<0.05$), while OXGR1 antagonist prevented PRR upregulation (0.79 ± 0.14-fold change of control).

Conclusion: Our results indicate that OXGR1 antagonism impacts on BP possibly by modulation of PRR expression.

Financing: FONDECYT 1220525

AREA: SKELETAL MUSCLE AND EXERCISE PHYSIOLOGY

P-68

Daily β-Hydroxybutyrate supplementation reduces intermuscular fat infiltration and accelerates muscle regeneration after an acute glycerol-induced injury in mice. A pilot study

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Introduction: Inflammation after injury can compromise muscle mass and functionality by inducing intermuscular adipose tissue (IMAT) infiltration. IMAT impairs regeneration, favoring a pro-inflammatory and metabolically altered muscle niche, leading to atrophy. Preventing IMAT via inflammation resolution may improve muscle regeneration. Ketone body β-hydroxybutyrate (βHB) has a potent anti-inflammatory effect, suggesting a potential use in muscle injury.

Objective: To examine whether βHB can prevent IMAT, thus promoting muscle regeneration in injured muscle.

Methods: Glycerol-induced muscle injury was used as a well-established mouse model to produce IMAT. Ethics was approved by INTA scientific ethics committee. 50 µl of 50% v/v glycerol (GLI) or saline (SAL) were injected into tibialis anterior (TA) of 14 ± 2w old male mice. During a 2-week recovery, 3 mg/g/day bw βHB or SAL were given via gavage (n = 18, 9 βHB, 9 SAL). Tissue decellularization, Oil-red O and H&E staining, adipogenic proteins and NLRP3 inflammasome were assessed in TA.

Results: Daily βHB supplementation markedly reduced IMAT in injured TA muscles (~42%; t-test $p=0.02$; Effect size [ES] 1.88).BHB administration reduced the protein content of perilipin-1, GLUT4, and NLRP3, whereas PDGFRα was increased (ES: 0.71; 0.82; 0.45, -0.47, n=6 CTL, 7 βHB). In addition, centrally nucleated fibers were significantly less in βHB compared with SAL (92.1 ±

6.5% vs 65.7 ± 7.3 %, p=0.02) and there was a right shift in the frequency of fiber CSA, indicating regenerated fibers.
Conclusion: These results suggest the role of βHB in reducing IMAT and favoring muscle regeneration after an acute injury.
Financing: MFO: SOCHINUT-Henry Nestle 2022 award.

P-69 ★*selected for oral communication*
Moderate-intensity constant and high-intensity interval training confer differential metabolic benefits in skeletal muscle, white adipose tissue, and liver of candidates to undergo bariatric surgery
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Introduction: Physical preparation is necessary to improve bariatric surgery outcomes, however, the ideal exercise prescription is unknown.
Objective: To compare the effects on skeletal muscle, white adipose tissue, and liver metabolism of a constant moderate-intensity training program (MICT) vs. a high-intensity interval training (HIIT) in candidates for bariatric surgery.
Methods: Study reviewed by Los Rios Scientific Ethics Committee (code 350/2020). 19 participants (17 women) were randomized into MICT (n=10, 50% of heart rate reserve (HRR) and/or 4-5/10 subjective sensation of effort (SSE)) or HIIT (n=9, 6 cycles of 2.5 minutes at 80% HRR and/or 7-8/10 SSE, interspersed by 6 cycles at 20% HRR). Both workouts consisted of 10 sessions on a treadmill. After training, samples of skeletal muscle (transversus abdominis), subcutaneous adipose tissue, and liver were extracted and analyzed to measure adiponectin, GLUT4, PGC1α, phospho-AMPK/AMPK, collagen 1 and TGFβ1. Mann-Whitney test was used for comparisons between groups (p<0.05-significant).
Results: MICT induced higher protein levels of PGC-1α in skeletal muscle (mean±SD: 1.1±0.27 vs. 0.7±0.4-fold-change, p<0.05). In the liver, MICT induced higher protein levels of phospho-AMPK/AMPK (1.0±0.37 vs. 0.52±0.22-fold-change) and PGC-1α (1.0±0.18 vs. 0.69±0.15-fold change; both p<0.05), while HIIT exhibited lower levels of collagen 1 (1.0±0.26 vs. 0.59±0.28-fold-change, p <0.05). In subcutaneous adipose tissue, higher adiponectin levels were found only after HIIT (1.1±0.48 vs. 1.9±0.69-fold-change, p<0.05).
Conclusion: MICT and HIIT generated differential metabolic adaptations in skeletal muscle, liver, and white adipose tissue, adaptations that suggest that exercise intensity in the pre-surgical stage of candidates for bariatric surgery generates differential metabolic adaptations.
Financing: FONDECYT 11200391.

P-70
Impact of HFpEF on skeletal muscle morphology and function in a mouse model
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Introduction: Patients with heart failure with preserved ejection fraction (HFpEF) exhibit diastolic dysfunction due to increased stiffness and decreased relaxation capacity of the ventricular wall. Despite being a cardiac condition, HFpEF patients experience decreased skeletal muscle size and strength, reduced exercise tolerance, and diminished quality of life.
Objective: To determine whether mice with HFpEF display morphological and functional alterations in skeletal muscles.
Methods: C57BL/6N mice were divided into a control diet (CD) group, a high-fat diet (HFD) group, and an HFD + L-NAME (1.5 g/L) (HFpEF) group for 20 weeks (Bioethics protocol: 22537–CQyF–UCH). Evaluations were conducted at the end of the treatment period. Mean ± SD, n=5-9 animals per group. Statistical analysis: ANOVA one-way. A value of p<0.05 was considered significant.

Results: HFpEF mice showed increased body and heart weight. Cardiac variables showed that treated animals exhibited a higher E/e´ ratio and developed exercise intolerance, indicating the development of HFpEF. At the skeletal muscle level, the gastrocnemius and quadriceps muscles had more weight in HFpEF animals than in other groups. However, these animals showed lower performance in strength and aerobic tests.
Conclusion: These results suggest that mice with HFpEF display skeletal muscle alterations that exacerbate exercise intolerance.
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P-71

Mitophagy/autophagy deregulation induced by botulinum toxin type A in the masseter muscle

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Introduction: Chewing disorders impact quality of life with high cost of diagnosis/treatment. Botulinum toxin type A (BoNTA) injection is widely used to induce paralysis of the masseter muscle, decreasing altered activity. However, in a preclinical model, we demonstrated that BoNTA injection induces masseter muscle atrophy, subsequently decreasing bone quality. It has recently been proposed that alterations in the mitophagy process (proper mitochondria turnover) are related to muscle atrophy due to denervation or inactivity. However, the role of this process in BoNTA-induced masseter atrophy is unknown.
Objective: To evaluate the expression levels of mitophagy/autophagy markers in mice masseter during atrophy evoked by BoNTA injection.
Methods: Unilateral injection of BoNTA (0.2U/10µl) was performed in the masseter muscle of adult-BALB/c-mice (IACUC-UChile #21446-ODO-UCH-e2). Autophagy/mitophagy markers were assessed in masseter muscles by immunoblot or immunofluorescence at 2-7d post-injection. Chloroquine (15mg/kg, *i.p.* every other day) was used for *in vivo* autophagy blockade. The results were expressed as mean±SEM (n=4-8; $p < 0.05$; t-test, one-way ANOVA, or Mann-Whitney).
Results: BoNTA significantly increased markers of autophagy (LC3 I-II and p62) and mitophagy (PINK1, Parkin, BNIP3) at 7d post-BoNTA. Strong staining puncta for LC3 were observed in masseter cryosections 7d post-BoNTA. Chloroquine injection did not prevent the increase in autophagy markers evoked by BoNTA. Moreover, an increase in the mitochondrial-mass marker TOM20 was observed.
Conclusion: The increase in mitophagy/autophagy markers evoked by BoNTA and no changes after autophagy blockade suggests that BoNTA is blocking the mitophagy/autophagy process in masseter muscle. Alterations in mitochondria turnover could be involved in muscle atrophy.
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P-72

Unraveling the influence of the ROS/TXNIP/NLRP3 pathway on glucose uptake in skeletal muscle during a diet-induced insulin resistance model

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Introduction: Recent evidence suggests the involvement of the NLRP3 inflammasome in skeletal muscle insulin resistance. In monocytes, the NLRP3 inflammasome is activated by a ROS-inducing interaction between TXNIP and NLRP3. However, the role of the ROS/TXNIP/NLRP3 pathway has not been reported during insulin resistance in skeletal muscle.
Objective: To investigate if the ROS/TXNIP/NLRP3 pathway plays a role in insulin resistance in skeletal muscle.
Methods: Male C57BL/6 mice were fed a high-fat diet (HFD) or a normal control diet (NCD) for 8 weeks. Samples from *flexor digitorum brevis* (FDB) muscle were obtained for analysis. Protein content was measured by Western blot analysis. ROS production was estimated by both malondialdehyde quantification and peroxiredoxin-2/3 dimerization assays.
TXNIP/NLRP3 interaction was assessed using the proximity ligation assay (PLA). NLRP3 inflammasome activity was estimated using the FLICA probe. Insulin-dependent glucose uptake was evaluated using the 2-NBDG uptake assay. Experiments included 3-8 animals per group. U-Mann Whitney and Kruskal-Wallis tests were used for comparative analyses. Procedures were approved by the University of Chile's Animal Care Committee (CBA 1143 FMUCH).
Results: Muscles from the HFD-group showed elevated NLRP3 content, increased ROS-dependent peroxiredoxin-2 dimerization, and higher malondialdehyde concentrations. Muscle fibers from the HFD-group exhibited more TXNIP/NLRP3 interactions and higher NLRP3 inflammasome activation. Incubation with N-acetylcysteine or the NLRP3 inhibitor MCC950 restored insulin-dependent 2-NBDG uptake in HFD-group fibers.
Conclusion: During insulin resistance, skeletal muscle displayed activation of the ROS/TXNIP/NLRP3 pathway. Blocking this pathway reinstated insulin-dependent glucose uptake, suggesting that the ROS/TXNIP/NLPR3 pathway is involved in the development of insulin resistance in skeletal muscle.
Financing: FONDECYT 1190406 & 1231103 (PLL) and Doctoral Fellowship ANID 21190269 (JR).

P-73

Signalling pathways, molecular functions, biological process and cellular components as a functional enrichment of a protein-protein interaction network of exercise and physical activity

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Introduction: It has been evidenced that exercise and physical activity can prevent and treat some diseases (Cardiometabolic, Neurodegenerative or Cancer). However, the effects of exercise and physical activity at the cellular or molecular level have not been fully identified.
Objective: To associate signalling pathways, molecular functions, biological processes, and cellular components as functional enrichment of a protein-protein interaction network of exercise and physical activity through automated data mining for the prediction of possible therapeutic effects at the cellular and molecular level.
Methods: The whole methodological process was automated with KNIME software and was divided into 4 bioinformatics activities: 1) Data mining in OPEN TARGETS PLATFORM and STRING to obtain the proteins associated with exercise and physical activity along with their interactions; 2) Determination of the most relevant proteins by topological value (degree, intermediation and closeness) using PYTHON programming language for statistical analysis; 3) Association of the top 10 resources as: signalling pathways, molecular functions, biological processes and cellular components of proteins, and 4) Identification of the relationship between the most relevant proteins with each resource.
Results: The achievement of these activities allowed us to obtain 9,215 proteins, and 12 proteins as the most relevant (CTNNB1, CREBBP, MAPK1, MAPK3, TP53, HRAS, UBA52, SRC, UBB, UBC, RPS27A and EP300), obtaining the association with their respective functional enrichments.
Conclusion: We were able to conclude that through automated data mining we can associate signalling pathways, biological processes, molecular functions and cellular components as functional enrichment of a protein-protein interaction network of exercise and physical activity.

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P-74

Impact of a centronuclear myopathy (CNM)-causing mutation in dynamin-2 on the mitochondrial morphology and function in myoblasts of a murine model of CNM

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Introduction: Dynamin 2 (Dyn-2) is a large GTP-ase required to membrane and cytoskeleton remodeling. Mutations in Dyn-2 cause Centronuclear Myopathy (CNM), a congenital skeletal-muscle disease. Growing evidence demonstrates that Dyn-2 participates in mitochondrial fission in different cell-types. Mitochondrial dynamics (fission and fusion) directly modulates mitochondrial function. It is still unknown whether Dyn2 modulates mitochondrial dynamics in skeletal muscles, or whether mutations causing CNM affect this process.

Objective: The aim of this study is to evaluate the impact of the p.R465W mutation in Dyn-2, the most common causing CNM, in mitochondrial dynamics and function in muscle cells.

Methods: A transgenic mouse harboring the **p.**R465W mutation in Dyn-2 was used a CNM-model following approved bioethical protocol (BEA 131-18 of 11180731 Fondecyt project). Their wildtype (WT) littermates were used as healthy controls. Myoblasts were cultivated from *Tibialis Anterior (A.T)* muscles of CNM and WT mice. Dyn2/DRP-1 colocalization was assessed to evaluate availability of Dyn2 in muscle cell-mitochondria. Mitochondrial size and morphology, as well as ATP-production were also analyzed. Resting and insulin-stimulated myoblasts were used. Data were expressed as mean ± SEM. Kruskal Wallis-test was applied to compare experimental conditions and $p<0.05$ was considered significant.

Results: The preliminary results show no significant differences in Dyn2/DRP1-colocalization, mitochondrial morphology or the ATP-assay in non-stimulated myoblasts. In both, WT and CNM-myoblasts, insulin enhanced Dyn2/DRP1-colocalization and ATP production.

Conclusion: At the myoblast-stage, the CNM-causing p.R465W mutation in Dyn2 does not affect mitochondrial dynamics and function. However, it must be evaluated whether something different occurs in mature skeletal muscle fibers.

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P-75

Fibrotic role of the CCL5 chemokine in skeletal muscle

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Introduction: Extracellular matrix (ECM) of skeletal muscle is key for muscle regeneration and strength transmission. A balance between the production/degradation of ECM components keeps its homeostasis. Some factors, such as TGF-β and CTGF, increment ECM components such as collagen-III, triggering its excessive accumulation and leading to fibrosis. CCL5 is a chemokine that induces fibrosis in the liver and heart. Skeletal muscle secretes CCL5 and expresses its receptors CCR1 and CCR5. However, the influence of CCL5 on muscle fibrosis is unknown.

Objective: Therefore, we will determine the fibrotic effect of CCL5 in skeletal muscle.

Methods: For that, Tibialis anterior (TA) muscle of C57BL/6 male mice (3-5 months old) was electroporated with a plasmid carrying the sequence of CCL5 to overexpress it. ECM accumulation and collagen-III were determined by Sirius red and indirect immunofluorescence, respectively. *In vitro*, we used primary myoblast and fibroblast obtained from the hind-limb muscles which were exposed to recombinant-CCL5 (rCCL5). Collagen-III protein levels were

analyzed by immunoblot. TGF-β mRNA levels were determined by RT-qPCR *in vivo* and *in vitro*. Unpaired t-tests were used for statistical analysis. UNAB bioethical committee 020/2022.

Results: CCL5 overexpression in TA muscle increased the ECM accumulation in 5%, collagen-III levels in 20%, and TGF-β expression in 4,5 fold. Myoblasts and fibroblasts incubated with rCCL5 augmented collagenIII protein levels in 1,6 and 3,4 fold, respectively, but only fibroblasts showed elevated levels of TGF-β mRNA (1,5 fold).

Conclusion: Therefore, CCL5 overexpression induced fibrosis in TA muscles probably with the high contribution of skeletal muscle fibroblast.

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Satellite cell function in CCLD-induced sarcopenia: unveiling the role of resistance training

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Introduction: Sarcopenia, characterized by muscle mass, strength, and function loss, is a significant concern, particularly when associated with chronic diseases like chronic cholestatic liver disease (CCLD). While the mechanisms of sarcopenia remain not fully understood, deregulated satellite cells (SC) are proposed in its development. Conversely, resistance training (RT) improves age-related primary sarcopenia. Nevertheless, the role of SC in CCLD-induced sarcopenia and the potential protective effects of RT remain unclear.

Objective: To evaluate the effect of RT on sarcopenia and the function of SC in muscles from CCLD mice.

Methods: C57BL6 Pax7^{CreERT2}; ROSA$^{mT/mG}$ male mice aged 3-4 months underwent CCLD induction with a hepatotoxin and a progressive RT. Strength, physical function, and muscle mass were evaluated. Also, protein levels and mRNA expression of sarcomeric proteins and myogenic regulatory factors were determined. The diameter of muscle fibers, SC fusion, and muscle regeneration were analyzed histologically. Primary culture from skeletal muscles was performed to assess the myogenic potential. Two-way ANOVA was used, and values correspond to the mean ± SEM (n=4-8). The Animal Bioethics Committee approved this work at UNAB (CEC21).

Results: CCLD led to altered SC function, evidenced by impaired fusion to muscle fibers, altered regeneration, deregulated SC-associated markers, and diminished myogenic potential. Remarkably, RT prevented CCLD-induced sarcopenia and mitigated these SC-related impairments (0.06±0.01 vs 1.49±0.14).

Conclusion: Our study revealed compromised SC function in CCLD-induced sarcopenia. Furthermore, our findings demonstrated that RT prevented CCLD-induced sarcopenia and restored SC function. In conclusion, our results highlight the potential of RT as a promising strategy.

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